PLANT PHYSIOLOGY

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ERRATA

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Page 17, table V, column 3, for first leader read 0.32.
Page 60, citation 40, for 885 read 855.
Page 82, line 13 from bottom, for "coworkers" read co-workers.
Page 98, citation 15, end of first line, insert "of".
Page 145, line 15, for "27.58" read 26.60.
Page 149, first author's name, for "Basslavskaya" read Baslavskaja.
Page 150, under Experimentation, B, for "NaCl" read NH_4Cl.
Page 169, table I, test B, transpose heading of last column.
Page 171, line 11, for "presure" read pressure.
Page 178, table IV, columns 4 and 5, transpose "shoots" and "roots" in headings.
Page 186, last line of text, for "0.385" read 0.395.
Page 205, second line from bottom, for initial "C" read L.
Page, 351, line 12 from bottom, for "response" read responses.
Page 411, citation 6, for "193" read 1933.
Page 436, citation 10, for initial "B" read R.
Page 454, first line, omit "C".
Page 484, table II, experiment B, last line of column 3, for "81.3" read 92.3.
Page 505, citation 15, for "Protoplasm" read Protoplasma.
Page 505, citation 16, for 6: 47–62. read 10: 121–146.
Page 506, citation 28, for "500–526" read 509–534.
Page 661, line 18 from bottom, for "pyhreliometer" read pyrheliometer.

PLANT PHYSIOLOGISTS AT THE SIXTH INTERNATIONAL BOTANICAL CONGRESS

AMSTERDAM, SEPTEMBER 2–7, 1935

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5. MRS. A. STOLL
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7. O. HAGEM
8. G. W. SCARTH
9. F. G. GREGORY
10. H. FITTING
11. W. STILES
12. F. E. LLOYD
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PLANT PHYSIOLOGY

JANUARY, 1936

THE SIXTH INTERNATIONAL BOTANICAL CONGRESS AT AMSTERDAM

WALTER F. LOEHWING[1]

(WITH PLATE ONE)

The Sixth International Botanical Congress at Amsterdam opened with a reception by the Netherlands Botanical Society in the commodious Koloniaal Instituut, Sunday, September 1, with approximately 1000 botanists in attendance. The first plenary session on Monday, presided over by Professor J. C. SCHOUTE, was addressed by the Minister of Education, who extended the hospitality of the Dutch Government, and by E. C. STAKMAN of St. Paul, who spoke on phytopathogenetic fungi. Monday, Tuesday, Thursday, and Saturday were given over to sectional meetings in agronomy, cytology, genetics, ecology, morphology, mycology, pathology, paleobotany, physiology, and taxonomy. Each section had selected a few special topics for consideration by several well known investigators representing different nations. These addresses were followed by liberal and efficiently conducted discussions, thus providing all participants with a helpful international viewpoint. Stenographic reports were made of all discussions and will be included with the papers in volume I of the Proceedings.

The agronomy section discussed interactions between plants, and experimental control of plant development. In cytology, separate sessions were devoted to chromosome structure, crossing-over, heteroploidy, and abnormal karyology. Addresses before the genetics section dealt with experimental changes of genotype, sexuality, breeding, and the rôle of cytoplasm in inheritance. Flower morphology and size in relation to form were the chief topics discussed in the morphology section. Pathology sessions centered on disease resistance, and biological forms of fungi. Other sections comprised paleo- and geobotany, mycology, and taxonomy. Topics discussed before the plant physiology section, under the chairmanship of Professor L. JOST, were photosynthesis, phytohormones, nitrogen metabolism, mineral nutri-

[1] Official delegate of the American Society of Plant Physiologists to the Congress.

tion, translocation, and cell wall structure. Dr. F. E. LLOYD's films on insectivorous plants were shown in a joint full congressional session Tuesday evening. The scientific program closed Saturday with an address by Dr. F. KÖGL of Utrecht on plant hormones and by Dr. BAAS BECKING of Leiden on the biology of the Zuiderzee. Stockholm was selected for the next international congress in 1940. The botanical section of the International Union of Biological Sciences met in joint session with the Botanical Congress Wednesday evening and passed certain resolutions concerning biological affiliations, government sanctions, and business policies at the next meeting.

The Physiology sessions were well planned, and enthusiastically received, daily attendance in this section averaging over two hundred. It was noted incidentally by the official delegate that 29 members of the American Society of Plant Physiologists attended the Congress. Special interest centered in the sessions devoted to translocation of plastic materials, and the cycle of development in plants.

Three important papers were read in the translocation program. Dr. O. F. CURTIS discussed the direction of solute transport through the phloem; Dr. T. G. MASON, in collaboration with E. PHILLIS, presented a paper concerning an activated diffusion theory of transport; and Dr. W. SCHUMACHER read a paper on the transport of fluorescein through the plant body.

The program dealing with the control of the cycle of development in plants contained two papers on yarovisation, one by Dr. A. RICHTER on yarovisation of grains and other plants, the other by Dr. P. S. HUDSON on vernalization, LYSSENKO's yarovisation. Photoperiodic control of the cereal grains was discussed by Dr. F. G. GREGORY, and Dr. F. E. DENNY presented an account of his work with chemical treatments of dormant plants. Dr. E. VAN SLOGTEREN dealt with the influence of various temperatures upon the development of flower bulbs.

The symposium on photosynthesis considered various angles of the problem. Dr. H. A. SPOEHR discussed some new approaches to the unsolved problems of synthesis, and Dr. D. BURK presented a paper on the kinetics of the BLACKMAN reaction. The state of chlorophyll in the plastids was discussed by Dr. L. G. M. BAAS BECKING. Other features of the photosynthesis discussion were papers on the relation of the chemical structure of chlorophyll and its function in the photosynthetic process by Dr. A. STOLL; on carbon dioxide concentration as a rate determining factor by Dr. R. EMERSON; and on the photosynthetic activity of the purple bacteria in relation to the general theory of photochemical reduction of carbon dioxide by Dr. C. B. VON NIEL.

Naturally the growth hormones received much attention, and the session devoted to them drew a large attendance. The hormones were considered as stimulators by Dr. H. FITTING; and Dr. F. W. WENT, whose work has con-

tributed so largely to our knowledge of growth hormones, discussed the hormones that are concerned in root development, and the phenomenon of inhibition. Seed hormones and the germination processes were analyzed by Dr. N. G. CHOLODNY; and Dr. H. SÖDING discussed the question of whether the growth hormone is unspecific. Substances causing cambial growth were considered by Dr. R. SNOW.

The structure of cell walls was discussed from the standpoint of growth, internal structure and swelling, and optical properties of cell walls. Oxidation-reduction phenomena were taken into consideration with reference to metabolism; and the synthesis of the amino acids, and the relation of secondary plant products to protein metabolism were discussed before the physiological section of the Congress. A few papers were read on the subject of permeability and accumulation of salts in cells. The nature of the limiting membranes, the mechanism of selective absorption, accumulation of salts by roots, and salt permeability and absorption in certain algae were the main contributions in this field.

In other sections of the Congress, plant physiologists found certain papers of interest. In the agronomy program, for instance, there were discussions regarding root activities, the mutual relations between plants and soils, and the contrasting behavior of legumes and non-legumes in nitrogen nutrition. It is regretted that a fuller account of these programs cannot be presented here, and that not all of the distinguished contributions can be listed.

All day excursions on Wednesday included visits to the botanical garden at Baarn, the late Professor WENT's Botanical Institute at Utrecht, floriculture laboratories at Lisse and Aalsmeer, the Rijksherbarium and Hortus Botanicus at Leiden, the laboratory for technical botany at Delft. On Friday excursions were made to the University College of Agriculture at Wageningen, and to the sand dunes on the Island of Voorne. Sunday, September 8, was reserved for trips to the Zuiderzee, while September 9 and 10 were devoted to plant stratification and dune studies at Valthermond and Drenthe. Final trips comprised inspection of the nurseries at Dedemsvaart, and a paleo-botanical conference at Heerlen.

The social program included a formal reception tendered to all participants by the Netherlands Government on Monday evening, September 2, at the Rijks-Museum where the famous illuminated display of Rembrandt's paintings had been arranged for the occasion. The sectional dinners on Thursday were preceded by a complimentary all-Congress steamer excursion through the city and harbor of Amsterdam, tendered by the municipality. Many special programs had been arranged for lady visitors. A formal banquet for all official delegates to the Congress was given at the Amstel Hotel after the final business sessions on Saturday night.

The Koloniaal Instituut was an ideal center for the Congress. Registration, mail, meetings, and meals were efficiently handled at the Instituut under the local committee headed by Misses A. C. SCHIPPERS and A. W. DEDEL while the scientific program was under the capable direction of Drs. J. C. SCHOUTE, O. DEVRIES, and M. J. SIRKS. An exhibition of publications, apparatus, colonial products, and Dutch scientific activity was held at the Instituut during the Congress. The organizing committee of the Congress has published a bulletin on The Netherlands as an Environment for Plant Life and another on Botany in the Netherlands, available through E. J. Brill and Co. of Leiden. Volume II of the Proceedings, containing abstracts of the papers given at the Congress, is available. Volume I containing the full reports of papers presented at the Congress is to be available in March. Communications relative to publications may be addressed to Professor M. J. SIRKS at the University at Groningen, Holland.

Every effort had been made to insure the success of the Congress. Both the federal and municipal governments contributed liberally to its support. The organizing committee not only arranged an excellent scientific program but provided abundantly for the convenience and entertainment of participants with the limited funds at its disposal. The success of the Congress is largely attributable to the indefatigable efforts and personal interest of the local committees. Informal luncheons and meetings for small groups might have facilitated greater personal contacts among workers in related fields, but the numerous events of the program preempted every available hour. It was regretted that conditions beyond the control of scientists prevented the attendance of the usual large delegation of Russian and German botanists.

STATE UNIVERSITY OF IOWA
IOWA CITY, IOWA

EFFECT OF AMMONIUM AND OF NITRATE NITROGEN ON THE COMPOSITION OF THE TOMATO PLANT[1]

HAROLD E. CLARK[2]

Introduction

Several recent studies (**6, 7, 38, 39**) of factors that affect the absorption and assimilation of ammonium and nitrate nitrogen by the tomato (*Lycopersicum esculentum* Mill.) have furnished observations on the problem of the metabolism of nitrogen in this plant. In addition, considerable information has been accumulated (**8, 9, 13, 16, 17, 18, 19, 20, 21**) concerning the influence of environmental conditions on its growth and composition. The experiments reported here are similar to those already mentioned in which the assimilation of ammonium and nitrate nitrogen has been studied but furnish new information concerning the composition of the plants. Improvements in analytical methods have made possible a more specific fractionation of the amide and amino nitrogen than heretofore, as well as the addition of the organic acids to the list of compounds in the tomato which are affected by the form of nitrogen absorbed.

The study was restricted to the composition of plants grown either with ammonium or with nitrate nitrogen. No attempt was made to investigate the conditions that affect the assimilation of either ion; a discussion of these factors is to be found in several recent papers (**1, 6, 7, 22, 24, 26, 27, 38, 39**). Two series of plants were grown at the same season during successive years, with closely similar results. For brevity, however, only the data of the second experiment are here presented.

Methods and materials

GROWTH OF PLANTS

Tomato plants of the Marglobe variety were grown from seeds which had been obtained from a single plant. The seeds were germinated in white quartz sand kept moist with tap water, and the seedlings were transplanted when about one week old to washed quartz sand in small clay pots. A dilute culture solution of the modified TOTTINGHAM series (**12**), $T_3R_1C_3$, previously found suitable for tomatoes (**5**), was applied to the sand daily after dilution with an equal volume of water. The dilute culture solution was prepared in tap water to which approximately 1 cc. of 1.0 N sulphuric acid per liter was added to give a pH of 4.8. Supplements of boron, manganese, and iron

[1] From the Biochemical Laboratory of the Connecticut Agricultural Experiment Station and the Osborn Botanical Laboratory of Yale University. A part of the expense of this investigation was borne by the Carnegie Institution of Washington.

[2] National Research Council Fellow, 1933–1935.

were added in the same forms and concentrations as those used in an earlier study (**6**). The complete solution, including both ammonium and nitrate nitrogen, was supplied to the plants for a month. At the end of this time the plants were about 16 cm. in height and had formed at least seven leaves.

From a group of 100 plants, 24 of uniform development were selected for growth in the experimental solutions. Eight plants were chosen for each treatment. The roots were washed free from adhering sand and solution, and were placed in sand that had been washed by prolonged agitation with fresh tap water until the supernatant liquid was no longer turbid. The sand used was a coarse grade of quartz obtained from Milltown, New Jersey.

The culture vessels were glazed stoneware crocks with a capacity of about 2.2 liters, furnished with a hole in the bottom to permit percolation of the solution. One plant was grown in each crock. The solution reservoirs, each of which supplied two cultures, were 6-gallon crocks provided at the bottom with stoppered outlets in which T-tubes were inserted, and were placed at a higher level behind the cultures. Capillary tubes of 0.5-mm. bore were attached to the arms of each T-tube by means of rubber connections closed with screw clamps which permitted regulation of the rate of flow of solution. Adjustments were made twice daily.

Three experimental solutions were utilized, the composition of each being given in table I. The ammonium solution designated as "concentrated" contained the same molecular proportion of nitrogen as did the nitrate solution. The "dilute" ammonium solution was included in the experiment because of earlier evidence (**38, 39**) of the adequacy of low concentrations of ammonium nitrogen for good growth of tomatoes.

The 0.5 M stock solutions of each of the several salts were prepared in distilled water, but tap water from the New Haven water supply was used in the preparation of all of the culture solutions. According to analyses kindly

TABLE I

COMPOSITION OF SOLUTIONS

SOLUTION	PARTIAL VOLUME-MOLECULAR CONCENTRATIONS				
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	$CaCl_2$	$(NH_4)_2SO_4$
Nitrate	0.0021	0.0042	0.0042	.....	.....
Concentrated ammonium	0.0021	.....	0.0042	0.0042	0.0042
Dilute ammonium	0.0021	.....	0.0042	0.0042	0.0014

furnished by the New Haven Water Company, this water was comparatively low in soluble materials, *e.g.*, 18 p.p.m. of calcium and 12 p.p.m. of sulphate having been reported in one analysis.

All of the solutions were adjusted to pH 6.7 by the addition of 0.68 cc. of 1.0 N potassium hydroxide per liter, in order to provide a favorable reaction for the absorption and assimilation of the ammonium ion (**6, 7, 38, 39**). The absorption and assimilation of the nitrate ion by the tomato are not so greatly affected by the pH of the medium (**6, 7**). The same supplements of boron, manganese, and iron were included as in the preliminary solution.

A group of plants was grown in each of the continuously renewed experimental solutions for 49 days. During this part of the growth period, the rate of renewal was increased from 1 to 3.6 liters per plant per day in an effort to maintain a constant pH in the culture solution flowing from the sand. A constant pH of 6.7 was not attained, however, a minimum level of pH 5.4 being reached in the case of some of the ammonium cultures about one week before the plants were harvested.

It was not possible to exclude nitrates completely from the ammonium cultures. Traces of nitrates, as indicated by the diphenylamine test on evaporated portions, were present in the tap water and also in the solution collected from the outlets of the vessels in which plants were supplied with ammonium sulphate.

A severe infection of leaf mold affected the lower leaves on all of the plants, and it was not until artificial heat was supplied in the greenhouse that the spread of the disease was checked. The infected leaves were removed from the plants, with the result that the lowest four leaves had been removed before harvesting of the series.

At least two blossom clusters had been formed on each plant by the harvesting date; all fruits were removed, however, soon after development began in order to avoid the effects of fruiting on the vegetative development of the plants (**14, 15**).

Preparation of tissue for analysis

The plants were harvested on October 29 and divided into three portions, roots, stems, and leaves. The stems were separated from the roots at the surface of the sand and the root portions were freed from sand by washing with water. The thick lower stem and transition region were discarded, and only the fibrous root system was preserved for analysis. Stem tissue samples included stems and all of the petioles and veins to which the leaf blades were not directly attached. Petioles and stems of tomatoes have been shown (**18**) to correspond closely in percentage and distribution of nitrogen other than nitrate. Leaf tissue was composed of the leaf blades and attached portions of veins or petioles. Leaflets with portions injured as a result of the growth of the leaf mold were separated from the healthy tissue and discarded after weighing.

The fresh weights of the tops of the individual plants were obtained before subdivision. The leaves were coarsely shredded, as were the roots, but the stems were sliced into sections 1–2 mm. in thickness. A period of about one hour was required to harvest the eight plants in each group and prepare them for the oven. A maximum loss of 5 per cent. of the fresh weight occurred during the weighing and shredding.

The preservation of plant tissue for subsequent analysis presents a problem for which no satisfactory general solution has been found. Preliminary experiments (**42**) indicated that it was not possible to use hot water extracts of the fresh tissues because of the hydrolysis of glutamine and liberation of ammonia that take place during the procedure. The use of cold water extracts (grinding with sand or ether cytolysis technique) was not practicable because of the impossibility of making all of the desired analyses within a short time on plants harvested simultaneously. Another alternative, freezing of the fresh tissue, was eliminated because of lack of facilities. The possibilities of a drying technique were therefore explored.

Root tissue of beets has long been known to contain large amounts of glutamine (**35**), which is readily hydrolyzed in water solution at elevated temperatures with the production of ammonia. Accordingly, drying experiments were conducted on this tissue and on sliced, green tomato fruits, with the object of testing the stability of glutamine in the tissue under various conditions (temperature) of drying. It was found that, under the conditions subsequently adopted, hydrolysis of glutamine, although appreciable, was not serious. Consequently this method of preparation was considered adequate for the purposes of the present experiment.

Each lot of tissue was dried rapidly at 80° C. in a Proctor and Schwartz drier. The oven was adjusted to give an air velocity of approximately 217 ft. per minute over the trays, and exhausted 43 cu. ft. per minute from the ports. Nearly all of the water was removed from the tissue within 30 minutes, but the material was dried at least three hours and was then cooled to room temperature, weighed, ground, and preserved in tightly stoppered bottles. All results of chemical analyses are expressed on the basis of this roughly dried material.

Analytical methods

Dry weight.—The percentage of dry matter in the fresh tissue was calculated from the initial dry weight obtained before grinding and bottling, and from the fresh weight of the minced tissue. The water content of the roughly dried tissue was determined by drying 0.5-gm. aliquots *in vacuo* over sulphuric acid to constant weight (96 hours); a decrease of only 1.4–2.8 per cent. occurred. The small amount of water present, the uniformity of the results of further drying, and the fact that no balance of solids was attempted,

made the use of the rough dry weight values adequate for the present purposes.

ASH.—The percentage of ash in the dried tissue was established by heating 0.5-gm. aliquots in a muffle furnace at a temperature of approximately 600° C. for 15 hours.

NITROGENOUS FRACTIONS.—Total nitrogen, including nitrates, was determined in appropriate aliquots of the dried tissue by the method of PUCHER, LEAVENWORTH, and VICKERY (**28**). Sodium thiosulphate was not added in the powder form but was dissolved in the concentrated alkali used.

Free ammonium nitrogen was obtained by moistening aliquots of the dried tissue with water and suspending the material in 30 cc. of water; 10 cc. of a 6.25 per cent. suspension of magnesium oxide were then added, together with a few drops of mineral oil, and distillation *in vacuo* at 40° C. for 15 minutes was carried out in an apparatus similar to that described by PUCHER, VICKERY, and LEAVENWORTH (**32**). Measurement of the amount of ammonia distilled was made in the manner described by these investigators.

Nitrate nitrogen was determined in ether extracts of the dried tissue secured according to the method of PUCHER, VICKERY, and WAKEMAN (**29**); nitrites, if present, would be included in this fraction.

Hot water extracts of the dried tissue were used for the determination of total soluble nitrogen, amino nitrogen, glutamine amide nitrogen, and asparagine amide nitrogen. These extracts were obtained by boiling appropriate aliquots in about 60 cc. of water for 3.5 minutes. The suspensions were then cooled, made to a volume of 100 cc., centrifuged, and the supernatant liquid filtered through a dry filter paper.

Total soluble nitrogen was obtained by the same procedure as that employed for total nitrogen in the dried tissue (**28**).

Total amino nitrogen was determined by means of the VAN SLYKE manometric gas apparatus according to the procedure outlined by PETERS and VAN SLYKE (23). Free ammonium nitrogen was first removed by distillation with the phosphate-borax buffer and borax-sodium hydroxide mixture used by PUCHER, VICKERY, and LEAVENWORTH (**32**). The residue from the distillation was neutralized with 2 cc. of 2 N acetic acid and made to a definite volume from which aliquots were taken.

Glutamine amide nitrogen was determined by a modification of the method of CHIBNALL and WESTALL (**4**), details of which are to be given in a forthcoming publication (**43**). The amide nitrogen of glutamine was doubled in order to give the total nitrogen of the compound.

Asparagine amide nitrogen was calculated as the difference between total amide nitrogen and glutamine amide nitrogen. Total amide nitrogen was obtained according to the procedure of PUCHER, VICKERY, and LEAVENWORTH

(**32**). The total nitrogen of asparagine is twice the amide nitrogen of the compound.

Insoluble nitrogen, the difference between total nitrogen and total soluble nitrogen, is referred to as "protein" nitrogen. There is some evidence (**3, 41**) that the greater part of the hot water-insoluble nitrogen of leaf tissue is protein nitrogen.

Total inorganic nitrogen was calculated by adding the respective values for nitrate and ammonium nitrogen. This is regarded for the most part as absorbed, but non-assimilated nitrogen, although both ammonium and nitrate nitrogen may arise from assimilated forms within the plant (**41**) under conditions favoring catabolism. Such conditions, however, probably did not prevail in these experiments because of the vegetative development of the plants.

Soluble organic nitrogen was calculated as the difference between the total soluble nitrogen and total inorganic nitrogen.

Amino nitrogen exclusive of that from glutamine and asparagine was calculated on the assumption that 85 per cent. of the total nitrogen of glutamine appeared as amino nitrogen in the procedure employed. The fact that not only the alpha-amino group but also the amide group of glutamine react with nitrous acid to form nitrogen gas, in contrast with the behavior of asparagine, has been observed before (**4, 35, 37**). In the VAN SLYKE procedure employed in this study, however, even pure glutamine did not yield gas equivalent to the total nitrogen of the compound. The factor of 85 per cent. was based on recovery experiments in which glutamine was added to extracts of tomato tissue. Amino nitrogen exclusive of that due to glutamine and asparagine was then calculated by deducting 85 per cent. of the glutamine nitrogen and 50 per cent. of the asparagine nitrogen from the total amino nitrogen obtained by direct analysis. Although the accuracy of this calculation is not established, the data are presented in order to bring out the apparent fact that the major portion of the amino nitrogen in the ammonium plants had its origin in glutamine and asparagine.

Known soluble organic nitrogen was calculated as the sum of glutamine and asparagine nitrogen and of the amino nitrogen exclusive of glutamine and asparagine.

Unknown soluble organic nitrogen was obtained as the difference between the total soluble organic nitrogen and the known soluble organic nitrogen.

ORGANIC ACIDS.—Total organic acids were determined by the method of PUCHER, VICKERY, and WAKEMAN (**30**). The use of the same factors as they employed for tobacco tissue was considered justifiable because citric, malic, and oxalic acids formed a large percentage of the total acids in the plants of the nitrate series. Although this was not true for the plants grown in the ammonium solutions, lack of information as to the nature of the acids present

in undetermined forms in these plants made any revision of the formula inadvisable.

Oxalic acid was determined according to their procedure (**30**). A reprecipitation of the calcium oxalate was found to be necessary in order to remove interfering pigmented material.

Citric acid and malic acid were determined by the method of PUCHER, VICKERY, and WAKEMAN (**31**). Those samples which included very small amounts of citric acid were analyzed by a modified method developed by PUCHER, SHERMAN, and VICKERY (**33**).

From these analyses the following fractions were calculated: (1) known acids, those determined by actual analysis, were obtained by adding the respective values for oxalic, malic, and citric acids; (2) unknown acids, those present in undetermined forms, were then calculated as the difference between the total organic acids and the known acids in each case; (3) the percentages of the total acids accounted for as known acids were also calculated and are included in the tables.

Experimental results

GROWTH OF PLANTS

Plants grown in the nitrate solution (hereafter referred to as nitrate plants) showed a more luxuriant growth than either of the groups of plants grown in the ammonium solutions (designated as concentrated ammonium plants or dilute ammonium plants, respectively). The leaves of the nitrate plants were somewhat lighter green in color and the total leaf area per plant was much greater than in the case of the ammonium plants. Furthermore the stems of the nitrate plants were of greater diameter than were those of the other groups. The average height of the nitrate plants was 111 cm., of the dilute ammonium group, 101 cm., and of the concentrated ammonium series, 87 cm. No relationship was noted between the number of blossoms or fruit formed and the cultural treatment.

The weights of the plants when harvested are given in table II. The average fresh weight of one plant was calculated from the eight members in each group. It is evident that the nitrate plants attained a much greater size than did the ammonium plants. The differences in average weight between the two groups of ammonium plants are not significant.

Table II also gives the percentage of the fresh weight composed of dry matter in each organ. The value listed for the leaves of the ammonium plants, 10.6 in each case, is an average figure calculated from the leaves of both groups, a procedure made necessary by an error in handling some of the aliquots. However, data from the earlier series of the preceding year, the similarity in composition of the two groups of ammonium plants, and the

TABLE II

Solution	Average fresh weight of one plant		Average dry weight of one plant			Dry weight expressed as % of fresh weight	
	Leaves	Stems	Leaves	Stems	Roots	Leaves	Stems
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	%	%
Nitrate	94.4	175.6	10.1	12.5	1.8	10.7	7.1
Concentrated ammonium	38.7	53.7	4.1	4.1	2.2	10.6	7.6
Dilute ammonium	37.6	69.1	4.0	5.6	1.5	10.6	8.1

perfect agreement in absolute dry weight values, all indicate that the value of 10.6 per cent. is very nearly correct.

The leaves of the three groups were nearly alike with respect to the amount of dry matter per unit of fresh tissue. A slightly higher percentage was found in the stems of the ammonium plants, especially in those of the dilute ammonium group, than in those of the nitrate series. The tomato plants grown in the nitrate solution probably should be classified as strongly vegetative, as their chemical composition with respect to nitrogenous fractions were similar to those described by Nightingale *et al.* (18) as "strongly vegetative, unfruitful plants," despite the fact that the respective percentages of dry matter were higher in his plants.

The average dry weights of the organs of each plant are also given in table II. Differences between the roots of the different plants may not be so significant as the data would indicate, inasmuch as some of the fine roots were lost during preparation of the sample.

Nitrogenous fractions

The influence of the form of nitrogen in the culture solution on the forms of nitrogen in the plants is evident from table III, which shows the several nitrogenous fractions in each group of plants expressed as percentages of the dry matter.

The total nitrogen in the leaves of the ammonium plants was greater per unit of tissue than in the leaves of the nitrate plants. This was due to a much greater concentration of insoluble or "protein" nitrogen, as well as of soluble organic nitrogen. Although somewhat greater concentrations of insoluble nitrogen and of soluble organic nitrogen were also found in the stems and roots of the ammonium plants than in the corresponding organs of the nitrate plants, the total nitrogen did not vary correspondingly because of the overbalancing influence of the presence of considerable amounts of nitrate nitrogen in the stems and roots of the nitrate plants.

TABLE III

NITROGENOUS FRACTIONS IN DRY TISSUES

FRACTIONS	NITRATE			CONCENTRATED AMMONIUM			DILUTE AMMONIUM	
	LEAVES	STEMS	ROOTS	LEAVES	STEMS	ROOTS	STEMS	ROOTS
	%	%	%	%	%	%	%	%
Total N	5.36	3.61	4.18	6.73	3.52	4.10	2.33	4.85
Insoluble or "protein" N	3.89	0.925	2.65	5.13	1.27	3.09	1.02	3.77
Total soluble N	1.47	2.68	1.53	1.60	2.25	1.01	1.31	1.08
Ammonium N	0.043	0.026	0.037	0.161	0.143	0.084	0.045	0.059
Nitrate N	0.390	1.68	0.621	0.019	0.050	0.029	0.114	0.052
Total inorganic N	0.433	1.70	0.658	0.180	0.193	0.113	0.159	0.111
Soluble organic N	1.04	0.979	0.876	1.42	2.06	0.895	1.15	0.971
Total amino N	0.300	0.240	0.158	0.610	1.22	0.397	0.555	0.360
Amino N exclusive of glutamine and asparagine	0.241	0.128	0.104	0.232	0.092	0.165	0.020	0.178
Glutamine N	0.038	0.110	0.044	0.278	1.13	0.210	0.476	0.146
Asparagine N	0.054	0.036	0.034	0.284	0.324	0.106	0.260	0.116
Known soluble organic N	0.333	0.274	0.182	0.794	1.55	0.481	0.756	0.440
Unknown soluble organic N	0.704	0.705	0.694	0.627	0.513	0.414	0.393	0.531

Total soluble nitrogen was higher in the ammonium leaves than in the nitrate leaves, but the opposite was true in the stems and roots, inasmuch as nitrate nitrogen was especially abundant in these organs of the nitrate plants. This high concentration of nitrate in the tissues is characteristic of the tomato plant when grown in a culture solution containing that form of nitrogen. Appreciable amounts of nitrate nitrogen were also found in the ammonium plants, presumably having been absorbed from the ammonium solutions, which were known to contain traces of nitrate. It is also possible that nitrate absorbed from the preliminary solution had not been completely assimilated. Still another possible explanation is the synthesis of nitrates under the proper conditions (**41**), to which reference has already been made.

Although the data for the quantities of ammonium nitrogen in all of the samples may be somewhat in error, owing to the drying technique employed in preparing the tissue for analysis, the constancy of the conditions under which the drying was effected was such as to render it probable that the results have relative value and that, within certain limits, valid conclusions may be drawn from them. High concentrations of ammonium nitrogen were found in the leaves, stems, and roots of the concentrated ammonium plants, in contrast with the low concentration in the corresponding tissues of the

nitrate plants. A high concentration of ammonium nitrogen in the solution apparently resulted in a high concentration in the plant. However, the absolute amount of ammonium nitrogen in the ammonium plants did not approach that of the nitrate nitrogen in the nitrate plants. Consequently total inorganic nitrogen was much higher in the nitrate than in the ammonium plants.

The relative assimilation of these two forms of nitrogen, indicated to some extent by the greater proportion of protein nitrogen in the ammonium plants, is also shown by the soluble organic nitrogen present in the three groups of plants. In all cases, the soluble organic nitrogen was much higher in the ammonium plants than in the corresponding tissues of the nitrate plants, despite the abundance of inorganic nitrogen, the potential source of organic nitrogen, in the nitrate plants. It is not likely, according to ECKERSON'S results (9), that the nitrate plants were deficient in reductase activity at the season when they were grown.

A consideration of the several fractions included in the soluble organic nitrogen in the different plants indicates the influence of the form and concentration of absorbed inorganic nitrogen on the compounds elaborated. The much higher concentrations of the acid amides, glutamine and asparagine, throughout the ammonium plants than in the corresponding tissues of the nitrate group were especially conspicuous. There was likewise more total amino nitrogen in the ammonium than in the nitrate plants.

However, if the amino nitrogen exclusive of that due to the presence of glutamine and asparagine is considered, the results appear in a different light. In the leaves, the net amino nitrogen values after the elimination of glutamine and asparagine did not differ significantly from each other, indicating that the original difference in total amino nitrogen was due almost wholly to the greater concentrations of these amides in the ammonium than in the nitrate leaves.

A similar distribution of amino nitrogen was evident in the roots and stems. Greater concentrations of total amino nitrogen in the ammonium than in the nitrate plants could again be accounted for by the presence of larger quantities of the amides in the ammonium plants. Amino nitrogen exclusive of glutamine and asparagine was greater in the ammonium than in the nitrate roots, but less in the ammonium than in the nitrate stems.

The amount of known soluble organic nitrogen was considerably greater in the ammonium plants than in the corresponding tissues of the nitrate plants, as might be expected from the large quantities of amino and amide nitrogen in the ammonium series.

Unknown soluble organic nitrogen, that present in undetermined forms, was uniformly higher in the nitrate plants than in the respective organs of the ammonium groups. The contrast between the nitrate and ammonium

series becomes more striking if the organic nitrogenous fractions are expressed as percentages of the total soluble organic nitrogen. In the nitrate leaves, 68 per cent. of the total soluble organic nitrogen was unknown, whereas the corresponding value for the ammonium leaves was only 44 per cent. In the nitrate stems, 72 per cent. of the total soluble organic nitrogen was unknown, in contrast to but 25 per cent. in the concentrated ammonium and 34 per cent. in the dilute ammonium series. Of the soluble organic nitrogen in the roots, 79 per cent. in the nitrate roots, 46 per cent. in the concentrated ammonium, and 55 per cent. in the dilute ammonium roots were present in unknown forms.

On account of the numerous fractions and several organs involved, a statement of the generalizations which can be made with reference to the assimilation of the two forms of nitrogen can be presented most clearly under separate headings.

SPECIFIC COMPOUNDS.—Plants grown in the ammonium solutions were characterized by higher concentrations of ammonium, glutamine, asparagine, and insoluble nitrogen than the nitrate plants. Those grown in the nitrate solution were distinguished by comparatively high concentrations of nitrate nitrogen.

FRACTIONS AS PERCENTAGES OF TOTAL NITROGEN.—Insoluble (or "protein" nitrogen) formed a relatively high proportion of the total nitrogen present in the ammonium plants as compared with the nitrate plants. Conversely, soluble nitrogen, and especially nitrate nitrogen, formed a larger proportion of the total nitrogen in the nitrate plants than in the ammonium groups. A larger percentage of the total nitrogen, nevertheless, was made up of soluble organic nitrogen in the stems of the ammonium groups than in those of the nitrate series.

DISTRIBUTION OF SOLUBLE NITROGEN.—Inorganic nitrogen, and its major component, nitrate nitrogen, were preponderant in the nitrate plants. In the ammonium plants, however, the larger part of the soluble nitrogen was organic.

DISTRIBUTION OF SOLUBLE ORGANIC NITROGEN.—Within this fraction much larger percentages of glutamine and asparagine nitrogen were found in the ammonium plants than in the nitrate series. As a result, total amino nitrogen and known soluble organic nitrogen composed large percentages of the soluble organic nitrogen in the ammonium plants. For example, known soluble organic nitrogen was as much as 75 per cent. of the total soluble organic nitrogen in the concentrated ammonium stems. In contrast, the major part of the soluble organic nitrogen in the nitrate plants was composed of unknown soluble organic nitrogen.

DISTRIBUTION OF NITROGEN WITHIN THE PLANT.—Calculated on the basis of an individual plant, the data bring out two points with reference to the

relative distribution of nitrogen between leaves and stems: (1) the ratio of nitrate nitrogen and, hence, of soluble nitrogen in the stems to that in the leaves was much greater in the nitrate than in the ammonium plants; (2) the ratio of amino nitrogen and, accordingly, of known soluble organic nitrogen in the stems to that in the leaves was considerably higher in the ammonium than in the nitrate plants.

Ash

The quantities of ash in the tops of the plants, expressed as percentage of the dry weight of the tissue in each case, are given in table IV. Inasmuch as the presence of traces of sand in the root tissue made it difficult to secure accurate data for this factor, no results from the roots have been included.

TABLE IV

Ash in dry tissues

Solution	Leaves	Stems
	%	%
Nitrate	17.2	20.7
Concentrated ammonium	12.7	14.0
Dilute ammonium	13.8	14.2

The influence of the cultural treatment on the ash of the tops was quite pronounced; both in the leaves and in the stems the percentages of ash in the dried tissue were much greater in the nitrate plants than in corresponding organs of the ammonium plants. No analyses were made for constituents of the ash, but it is probable, in view of the results of Stewart, Thomas, and Horner (**36**) with pineapples, of Prianischnikow (**25**) with beets, of Holley, Picket, and Dulin (**10**) with cotton, and of Jacobson and Swanback (**11**) with tobacco, that a greater absorption of calcium and of other bases took place from the nitrate than from the ammonium solution.

Organic acids

One of the most pronounced contrasts between plants of the ammonium groups and those of the nitrate group was in the concentration of organic acids in the tissues. The results, expressed as percentages of the dried tissue, are given in table V. It is evident that the concentrations of the individual organic acids were all higher, respectively, in the nitrate than in the ammonium plants. Consequently, the sum of the three acids, or the known acids, was higher in the nitrate group. The differences were extraordinarily large in most cases.

In this connection, it is of interest to recall that Ruhland and Wetzel

TABLE V

ORGANIC ACIDS IN DRY TISSUES

SOLUTION	ACID	LEAVES	STEMS	ROOTS
		%	%	%
Nitrate	Oxalic	2.12	3.07	0.91
	Malic	2.39	2.61	0.56
	Citric	1.78	0.70	0.53
	Known acids	6.29	6.38	2.00
Concentrated ammonium	Oxalic	0.12	0.43	0.02
	Malic	0.15	0.33	0.24
	Citric	0.05	0.08	0.09
	Known acids	.....	0.84	0.35
Dilute ammonium	Oxalic	.....	0.72	0.03
	Malic	.....	0.56	0.55
	Citric	.....	0.09	0.09
	Known acids	.....	1.37	0.67

(34) found that *Begonia* leaves grown in a nitrate solution contained more oxalic acid than those grown in an ammonium solution.

The values in table VI are presented in order to make comparisons on an equivalent basis. The milliequivalents of each acid in 100 gm. of dry tissue

TABLE VI

ORGANIC ACIDS IN DRY TISSUES

SOLUTION	ACID	MILLIEQUIVALENTS IN 100 GM. OF DRY TISSUE		
		LEAVES	STEMS	ROOTS
Nitrate	Oxalic	47.1	68.2	20.2
	Malic	35.7	39.0	8.4
	Citric	27.8	10.9	8.3
	Known acids	110.6	118.1	36.9
	Total acids	153.0	147.0	98.0
	Unknown acids	42.4	28.9	61.1
	Percentage known acids	72.1	80.6	37.7
Concentrated ammonium	Oxalic	2.7	9.6	0.4
	Malic	2.2	4.9	3.6
	Citric	0.8	1.3	1.4
	Known acids	5.7	15.8	5.4
	Total acids	71.0	65.4	50.9
	Unknown acids	65.3	49.6	45.5
	Percentage known acids	8.0	24.2	10.6
Dilute ammonium	Oxalic	.....	16.0	0.7
	Malic	.....	8.4	8.2
	Citric	.....	1.4	1.4
	Known acids	.....	25.8	10.3
	Total acids	.....	73.8	80.4
	Unknown acids	.....	48.0	70.1
	Percentage known acids	.....	35.0	12.8

have been calculated, as well as the milliequivalents of total acids. Likewise unknown acids (the difference between total acids and known acids in each tissue) are expressed on the same basis. The percentages of total acids accounted for as known acids are also listed.

These data show that both the known and the total acids were much more abundant in the nitrate than in the ammonium plants. Moreover, the percentage of the total acids composed of the three known acids was much greater in the nitrate plants than in the corresponding organ of the ammonium plants.

On the other hand, unknown acids not only formed a large fraction of the total acids in the ammonium plants, but also were present actually in larger amounts per unit of tissue in the ammonium than in the nitrate plants, with the single exception of the roots in the concentrated ammonium series.

Discussion

In general, the differences between the concentrated ammonium plants and the dilute ammonium plants were differences in degree apparently influenced by the difference in concentration of ammonium nitrogen in the solutions. The height of the plants, the average weight of the respective organs, the percentage of dry matter in the several tissues, and the concentration of specific compounds or fractions within tissues of the dilute ammonium plants were, in most instances, intermediate between the respective values obtained from the concentrated ammonium group and the nitrate series.

In other words, the magnitude of the difference between tomatoes grown in solutions including the same concentration either of ammonium or of nitrate nitrogen was diminished when the concentration of ammonium nitrogen in the solution and, consequently, in the plant was decreased. Significant exceptions to this generalization were largely confined to the roots and to those fractions not including specific compounds or compounds of a similar nature, *e.g.*, total, total soluble, and unknown soluble organic nitrogen.

This relationship between the concentration of ammonium nitrogen in the plant and its growth and composition indicates the fundamental importance of this form of nitrogen in relation to protein synthesis and growth. The data are not sufficiently complete to warrant an assertion that the unfavorable effect of a high concentration of ammonium nitrogen in the tissue was due to any "toxic" effect of this ion, *per se*. It is equally probable that lower concentrations of ash, of organic acids, or of other constituents in the ammonium than in the nitrate plants were the causal factors related to the lesser growth of the ammonium groups.

Furthermore, the importance of the acid amides, glutamine and as-

paragine, in the metabolism of the tomato is evident. It is clear that they are synthesized in larger amounts when larger quantities of ammonium nitrogen are present in the tissues. Yet the data at hand do not justify any conclusion as to the relationship of these amides to protein synthesis.

The uniformly large proportion of soluble organic nitrogen present in undetermined forms in all tissues of the nitrate plants raises a question as to the importance of this unknown nitrogen, especially in view of the greater growth of the nitrate plants. On the other hand, this fraction, or specific compounds therein, may have accumulated more in the nitrate plants than in the other groups as a result of a greater abundance of the precursors of the non-nitrogenous parts of the molecules in the nitrate plants.

It is clear that the organic acids are active in the metabolism of the tomato, but their exact function remains obscure. As pointed out by VICKERY and PUCHER (**40**) and by BENNET-CLARK (**2**), several precursors and several modes of decomposition have been suggested, none of which have been fully established. In the data presented, low concentrations of the organic acids in the ammonium plants may have been related to the supply of carbohydrates in the plants. Unfortunately these data have not been obtained. It may be, however, that the amides in the tissues were formed at the expense of the organic acids (or their precursors), with consequent reduction in the amounts of organic acids present in tissues rich in amides.

In addition, the fact that the nitrate plants included not only larger amounts of organic acids, but also of ash, than did the ammonium groups suggests a correlation between the organic acids and ash. That no quantitative relationship could be established is not surprising, for both inorganic and organic acids and bases should enter, theoretically, into the acid-base balance within the plant. Furthermore the possibility exists that the nitrate ion was accompanied by a cation when absorbed from the solution, whereas the ammonium ion was accompanied by an anion. Such phenomena would help to account for the differences in concentrations of ash observed.

As a whole, the data reported here serve to emphasize the dual nature of the influence of the form of nitrogen in the solution on the metabolism of the plant. In the first place, there is the absorption of the ion itself, with resultant effects upon the absorption and accumulation of other ions. In the second place, there are the effects of the concentration and form of inorganic nitrogen within the plant upon its composition and growth. Furthermore the results show that the absorption and assimilation of nitrogen, the accumulation of ash constituents, and the formation of organic acids in the tomato plant are interrelated processes.

Summary

1. Tomato plants one month old were subsequently grown for 49 days in sand culture with continuously renewed solutions at pH 6.7. One solution

contained only calcium nitrate as a source of nitrogen; another, an equivalent amount of ammonium sulphate; a third contained one-third as much ammonium sulphate. Analyses of the dried tissue of the harvested material showed the influence of the form and concentration of nitrogen in the solution upon the growth and composition of the plants.

2. Differences between the nitrate plants (those grown in the nitrate solution) and the ammonium plants (those grown in the ammonium solutions) were diminished by decreasing the concentration of ammonium nitrogen in the solution.

3. The fresh weight of the tops was greater in the nitrate than in either of the ammonium series. There was no significant difference in weight between the plants grown in the dilute and those in the concentrated ammonium solutions.

4. The dry matter, expressed as a percentage of the fresh weight, was slightly higher in the ammonium than in the nitrate stems, but the dry matter in the leaves of each series was practically the same.

5. A high concentration of nitrate nitrogen was found in the nitrate plants; consequently total inorganic or unassimilated nitrogen was much higher in the nitrate than in the ammonium plants.

6. A considerably greater concentration of ammonium nitrogen was found in the ammonium than in the nitrate series. Furthermore there was a greater accumulation in the plants grown in the concentrated than in those grown in the dilute ammonium solution.

7. Much greater quantities of glutamine and of asparagine nitrogen were present in the ammonium than in the nitrate plants, an indication of the ready formation of amide from ammonium nitrogen.

8. More total amino nitrogen was found in the ammonium plants than in corresponding tissues of the nitrate group, but this was due largely to the greater concentration of glutamine and of asparagine in the ammonium series. Amino nitrogen exclusive of that from glutamine and asparagine formed a small part of the total amino nitrogen in the ammonium plants, but a relatively large part in the nitrate group.

9. A greater concentration of soluble organic nitrogen was found in the ammonium than in the nitrate plants. In the ammonium series, moreover, the larger portion of this was known soluble organic nitrogen, *i.e.*, that present in forms analytically established. In contrast, more unknown soluble organic nitrogen, that present in undetermined forms, was found in the nitrate than in the ammonium plants. Indeed the larger part of the total soluble organic nitrogen in the nitrate plants was unknown.

10. The concentration of insoluble or "protein" nitrogen was greater in the ammonium than in the nitrate plants.

11. The percentage of ash was higher in the nitrate than in the ammonium plants.

12. The concentration of the three known organic acids, oxalic, malic, and citric, respectively, was much greater in the nitrate series than in the corresponding tissues of the ammonium plants.

13. The quantity of total organic acids was also greater in the nitrate than in the ammonium series. Of the total acids present in the tops of the nitrate plants, the known acids constituted the greater portion. On the other hand, unknown acids, those present in undetermined forms, composed the larger part of the total acids in the ammonium plants and in the roots of the nitrate series. Unknown acids, with one exception, were present in greater concentrations in the ammonium plants than in corresponding tissues of the nitrate plants.

14. An interrelationship is suggested between the absorption and assimilation of nitrogen, the accumulation of ash constituents, and the synthesis of organic acids by the plants, evidence of the complexity of the effect of the form of nitrogen in the culture solution.

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CONNECTICUT AGRICULTURAL EXPERIMENT STATION
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SEASONAL STUDY OF TISSUE FUNCTION AND ORGANIC SOLUTE MOVEMENT IN THE SUNFLOWER[1]

OLIVER A. LEONARD

(WITH SIX FIGURES)

Introduction

Studies of seasonal changes of the nitrogenous and carbohydrate fractions of many plants have been made, but in few cases have the plants been sufficiently divided to show the functions of individual tissues. Usually when detailed work has been done, not all parts have been analyzed. Of course, many plants are constructed so as to make subdivision difficult and impractical. Hence, in this study it was decided to use the common cultivated sunflower (*Helianthus annuus* L.) as it fulfilled the requirement of being easily divided into anatomically distinct regions.

Some work has already been done on various tissues of this plant over short periods. CLEMENTS (**12**) has dealt with the hourly variations of carbohydrates in the leaves. He has likewise studied seasonal changes in the sunflower, and although these data have not been published, they will be referred to from time to time in this paper.

The purpose of this work was to determine the functions of plant tissues during various stages of growth and to obtain information of the synthesis and translocation of organic solutes.

Literature

METHODS OF CALCULATION.—There appears to be considerable controversy over the method of reporting analytical results. Thus one finds data recorded as percentages of green weight (**12**), dry weight (**32**), residual dry weight (**28**), water weight (**28**), and unit area (as in leaves) (**29**), or unit organ (**41**). None of these bases is satisfactory. When comparing different organs for soluble products, it would seem as though the water basis is at present the most satisfactory. Yet it is known that even within a given cell there is considerable variation in the concentration of the various solutes in water. For changes in a given organ, residual dry weight seems to be at present the best basis. Here again, however, this is known not to be constant. In *Echinocactus* which was stored for six years in a room with diffused light, MACDOUGAL (**26**) noticed that the residual material decreased in the same proportion as the other carbohydrates. If soluble substances are translocated along colloidal surfaces (**13**) or in response to colloidal activity, it would seem as though percentages should be based on the amount of proto-

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plasmic colloids active in this respect. The entire problem as to the basis upon which results should be reported is yet to be worked out.

CARBOHYDRATES.—SPOEHR (**38**) found in the cactus (*Opuntia phaeacantha*) that the greatest growth activity comes when the monosaccharides and disaccharides are highest; this was in the spring just before blooming. Likewise THOMAS (**43**) found in the apple tree that an accumulation of "glucose" and sucrose appears to be a necessary precursor to growth. Active growth resulted in a decrease in the "glucose" and sucrose but these accumulated again as growth acceleration diminished.

After studying the cactus, SPOEHR (**38**) made the following generalizations: "Low water content and high temperature are associated with: (1) increase of polysaccharides; (2) decrease in monosaccharides; (3) increase in pentosans. High water content and lower temperature are associated with: (1) decrease of polysaccharides; (2) increase of monosaccharides; (3) decrease of pentosans."

NEWTON'S work (**31**), showing the accumulation of sugar during the late fall and winter for winter wheat and the frequently noticed anthocyan accumulations in the cold, supports SPOEHR'S conclusions. HARTT (**24**) likewise has noticed that an increased moisture percentage in the sugar cane favors hydrolysis by invertase rather than synthesis. However, near freezing temperatures hardening is brought about, this being associated with increased amounts of acid-hydrolyzable material. Thus the various relationships are quite complex.

PARKIN (**34**) and others have noticed that the ratio of sucrose to hexose is greatest in the leaves early in the season, while later the reverse is true; in other words, the large sucrose/hexose ratio is associated with young leaves, and the small sucrose/hexose ratio with old leaves. Hence it appears as though invertase activity is greater and more sensitive to solution changes in the young leaves than in the old.

Acid-hydrolyzable material appears to perform a nutritional function similar to starch, except under drought conditions (**12**). This material represents a wide group of substances which have different physiological effects. CLEMENTS found that under drought conditions the acid-hydrolyzable material in the leaves fluctuated very little during a day. This indicates that these substances are not functioning as food reserves, but as protective materials. During seasons or parts of seasons when drought conditions were absent, these substances fluctuated markedly in the leaves during the day, thus indicating that they may act as food reserve material.

It is now rather well agreed that the simple sugars are the first sugars of photosynthesis (CLEMENTS (**11, 12**), BARTON-WRIGHT (**4**), HARTT (**23**), SPOEHR (**37**), MASON and MASKELL (**28**), and others); while in the older works BROWN and MORRIS (**6**), PARKIN (**34**), DAVIS, DAISH, and SAWYER

(17), and others believed sucrose was the first sugar. Even in the older works there was some support of the simple sugar hypothesis (STRAKOSCH (40), CAMPBELL (9), and others). The possible lack of sucrose in some algae would tend to support this idea (2). Recently CLEMENTS (11) excluded sucrose, mannose, galactose, and pentoses as the primary sugar on the basis that they are not always present in photosynthetic tissue, and pointed out that so far as observed facts are concerned, fructose and glucose must together be regarded as the first sugars.

After the primary sugar is formed, it is readily converted into other forms (4, 12, 28). SPOEHR (37) summarizes our present position on the fluctuations in carbohydrates in photosynthetic tissue by stating, "Thus we are dealing with an exceedingly complex 'dynamic equilibrium,' the changes in which are brought about by conditions which are as yet imperfectly understood." ECKERSON (19) has shown the variability of pH in cells, while SCARTH (36) has shown pH to be highly variable and important in the interconversion of carbohydrates. CLEMENTS (13) has reviewed the subject of translocation of organic solutes in plants. The colloid content of the phloem cells is very high, much higher than in other plant tissues, which indicates that the movement of organic solutes may be due to certain colloidal responses. He suggests that the organic solutes may move along colloidal surfaces, since CRAFTS (16) has shown that it is probable that protoplasmic strands are continuous throughout the plant. STEWARD (39) suggests that energy from living protoplasm assists in the movement of organic solutes, a suggestion which HOAGLAND (25) originally made for the inorganic solutes. It is possible that there is a gradient from "source" to "sink" on or near the colloid surfaces. The amount of any particular organic solute concentrated in the interface depends on the nature of the solute, the colloid surface, and the solution. Since this is the case, conclusions cannot be drawn from gross macrochemical analyses as to the gradients existing on these surfaces.

BARTON-WRIGHT (3) found sucrose in the stems of healthy potatoes and that the concentration in the lower stem was less than in the upper. From this he concluded that sucrose was the translocatory sugar in the normal potato. CLEMENTS (14), for a period of from four to six weeks midway in the development of the potato, found no sucrose present in the stem, but large simple sugar concentrations. In another case he found sucrose was absent in sunflower petioles during the day until 4 P. M. and then present only in very slight amounts. Working with potatoes, WERNER (49), on a green weight basis, found that under plus N conditions there may be either positive or negative sucrose gradients from the upper to the subterranean stems, while the simple sugar gradients are always positive. Under minus N conditions, sucrose formed decidedly negative gradients (as for example upper stem 0.162, lower stem 0.227, subterranean stems 0.428, tubers 1.021 per cent.).

The simple sugar gradients were positive (upper stem 0.244, lower stem 0.159, subterranean stems 0.121, tubers 0.121 per cent.). BULGAKOVA *et al.* (7) reported that sugar beets grown under electric lights of average strength and those grown in sunlight showed maximum monosaccharides in the petioles, slightly less in the veins, still less in the parenchyma, and least in the roots. Sucrose increased continuously from the leaf parenchyma to the roots. In plants two months old or older, these conditions remained unchanged at all hours of the day and night. They believed that monosaccharides were moved in that form from the leaf parenchyma to the roots.

At present there are no data available which would militate against the idea that both sucrose and the simple sugars move together. The gradients which have been obtained appear to be greatly influenced by the conditions under which the plants were grown. The relative proportions of the simple sugars to sucrose probably represent stages in a dynamic equilibrium between these sugar forms. At present the only thing which is relatively certain is that reducing sugars may be the only sugars of translocation, since sucrose is sometimes entirely absent from potato stems or sunflower petioles (**12**). This may mean that the simple sugars are always translocatory sugars. From the large amounts of sucrose which may occur in different bark sections (**28**) it would seem likewise possible for sucrose to be an additional motile form. It is probably true that both the simple sugars and sucrose together are active in translocation, and that in the absence of either, the other may act in this capacity (**13**).

NITROGEN

The problem of the seat of synthesis of organic nitrogen from the inorganic has interested plant physiologists for some time. At first it was considered that organic nitrogen was synthesized chiefly in the leaves. Later THOMAS (**42**), NIGHTINGALE (**32, 33**), TIEDJENS (**45, 46, 47**), ECKERSON (**19**), and others found that synthesis may take place largely in organs other than the leaves. MASKELL and MASON (**27**) believed that in the cotton plant the inorganic is transformed to the organic chiefly in the leaves, although their evidence is not complete.

The change of nitrate to organic nitrogen appears to take place in the presence of reducase (**20, 21**) along with a proper pH, carbohydrate, and PO_4 ions. In many plants reducase is present in many parts, but reduction apparently takes place in those regions where the nitrate first comes into contact with proper conditions. Thus in asparagus (**32**), all parts contain reducase, but synthesis takes place in the fine roots. If the temperature is lowered to 10° C., however, nitrates migrate to all parts and when the temperature again is raised to normal (20°–30° C.), nitrates are reduced and synthesized to organic nitrogen in all parts. THOMAS (**42**) found nitrates

were entirely reduced in the fine roots of the apple tree and synthesized to organic nitrogen, even though many other parts of the tree contained reducase, as has been shown by ECKERSON (**22**). If reducase activity is not very high in those regions where the nitrates first enter, only a portion of them will be reduced; the same might be brought about by a very rapid absorption of nitrates, furnishing more substrate in transit than can be acted upon by the enzyme. TIEDJENS (**46**) suggested that if the most favorable pH for nitrate absorption had been used by THOMAS (**42**), probably he would have found nitrates present in some aerial parts.

ECKERSON (**22**) found that for each plant there is a characteristic distribution of reducase in the organs; in some it was present in the tops (soy bean), in some in the roots (asparagus), and in some both (tomato). Likewise she has shown that reducase activity is markedly affected by nutrition, light intensity, photoperiod, temperature, and is subject to a differential seasonal change in different organs.

In nitrogen starved tomato plants ECKERSON (**19**) traced the process of nitrogen assimilation microchemically. It is of considerable significance that she found the phloem parenchyma and inner cortical tissue as being regions carrying on reduction. Starch disappeared from cells having an acid pH and in its place appeared organic acids, such as aspartic, alanine, leucine, cystine, histidine, and other unidentified forms, and the amide asparagine. PRIANISCHNIKOW (**35**) concluded that ammonia is the first as well as the last stage of protein synthesis. Using etiolated plants he found that light was unnecessary for amino acid formation provided plenty of carbohydrate was present.

Nitrates may accumulate in the soy bean (**48**), wheat (**18**), *Amaranthus*, and other weeds (**8, 50**) up to and during flowering, and then decrease as they are transformed into organic nitrogen and used in the developing seeds and fruit. Nitrates may be absorbed by plants over a wide pH range without ill effects, but this is not true of ammonia or of soluble organic nitrogen. If ammonia is absorbed at a pH of 4, the plant is very definitely harmed. Apparently plants have become physiologically adapted to the use of nitrates (**14**). The stored nitrate nitrogen insures an easily accessible nitrogen supply that can be used as needed.

MASKELL and MASON (**27**) believe that nitrates and ammonia are transformed to organic nitrogen chiefly in the leaves, and are translocated on patterns similar to diffusion. However, except for "residual nitrogen" their soluble organic nitrogen gradients are negative. Their data do not seem to support their view that the leaves of the cotton plant are necessarily the chief tissue synthesizing organic nitrogen. Diurnal variations in total and soluble nitrogen have been used as evidence of synthesis of organic nitrogen by leaves (**10, 27**).

Considering the various available data, the proportion of nitrogen assimilated in the leaves and in other parts undoubtedly varies with different plants and the conditions of growth.

THOMAS (**43**) found that leaves of the apple tree decrease in total nitrogen before and during the time of chlorophyll disintegration, while the other tissues, especially the short spurs, markedly increase. Since the lower leaves of the sunflower begin to die long before the upper leaves, there is a migration of nitrogen out of these parts into others. CLEMENTS (**14**) found, as also did the writer, that the total nitrogen per sunflower plant was considerably higher when the heads were still small (containing about one tenth as much nitrogen as they did at maturity) than at the end of the season. The decrease in total nitrogen from the entire plant was caused by leaf fall, while the increased nitrogen of the head was a result of its movement from the dying leaves and maturing other parts as well as a continued synthesis from the inorganic nitrogen. The final piling up of amino acids as the seeds matured was due to their continued formation after the "sink" was no longer available. Somewhat similar results were obtained by WEBSTER (**48**) for the soy bean.

The variations of the nitrogen curves are closely dependent upon environmental conditions, as CLEMENTS (**15**) has shown with water culture experiments. THOMAS (**44**) found the addition of nitrate or its absence resulted in different curves; while TIEDJENS (**46**) by adding ammonia under optimum as well as adverse pH concentrations caused widely different degrees of assimilation. Light, temperature, potassium, phosphorus, and other elements produce profound effects upon the relative abundance of these different nitrogen fractions.

The relationship between amino acids and amides often appears to be linked with the relative abundance of ammonium nitrogen. Thus high ammonium concentrations may cause a conversion of amino acids to the amide. However, the relative abundance and effects of specific enzymes may largely determine these reactions. TIEDJENS (**45**) found that under conditions of high ammonium concentrations a part of it was loosely combined. These may have formed ammonium salts of amino acids, organic acids, etc., or may have been combined or adsorbed by colloidal surfaces. ECKERSON (**19**) observed that there were large accumulations of asparagine in all young growing regions of the wheat plant and believed it to be an important nutritive substance for growth.

Materials and methods

Although the work presented in this paper represents data from two seasons of collections, the second year's work will be referred to and used for the most part. Each year an acre of the common cultivated sunflower was grown on the Leonard farm, 3 miles from the laboratory. The soil upon

which the plants were grown was a rather uniform Palouse silt loam located on a south slope, containing ample moisture for good growth. The plot for the first year contained ordinary amounts of soil salts and moisture, while that for the second year was located near the bottom of a small cove, and apparently contained large amounts of soil salts and an abundance of moisture.

The material for the seasonal collections was gathered once each week between four and five o'clock in the morning. Early in the season approximately forty plants were collected, but toward the end this was reduced to ten. The plants were taken to the laboratory where they were subdivided into the different parts. The roots were removed at the base of the hypocotyl, washed, dried with paper towels, and weighed. Early in the season the stems were divided into two parts, later into three, and finally into four. In all cases the lower section consisted of the hypocotyl and the first two internodes. The second section consisted of the rest of the stem for the twice divided stems and of the third, fourth, and fifth internodes for the stems divided into three or four parts. The third section consisted of the rest of the stem for the stems divided into three parts and all but the upper 2½ feet for the stems divided into four parts. All of the upper sections were divided into bark-and-wood as one sample and pith as the other. The rest of the stem sections were separated into bark, wood, and pith. The terminal buds were analyzed separately at first, but as soon as these developed sufficiently, the fruits and the rest of the head, made up of receptacle and bracts, were analyzed separately. Healthy leaves collected below the uppermost 1 foot were called "old leaves," while any of the leaves contained within the upper 1 foot were called "young leaves." The samples used ranged from 100 to 600 gm. The carbohydrate material was placed in a constant temperature oven kept at 65° C., while that for the soluble N fractions was autoclaved for 15 minutes under 15 pounds pressure.

The young leaves for the hourly series were collected from the same part of the plant as in the seasonal series, but the old leaves were collected immediately below the upper third of the plant. These were rushed to the laboratory where the petioles were removed. The soluble N material and the carbohydrate material were immediately autoclaved. Ninety-five per cent. ethyl alcohol made slightly alkaline with NH_4OH was poured over the carbohydrate material and stored away for analysis. Three hundred gm. of material were used for soluble N samples, 200 for carbohydrates, and 100 for the moisture determinations. At the same time the samples were gathered, readings of the light intensity, temperature, and relative humidity were made. The Friez hygro-thermograph was used for determining the temperature and relative humidity of the atmosphere. The light intensity was measured with a Weston illuminometer.

MOISTURE.—The moisture content of materials was determined by allowing them to remain in the drying oven at 65° C. for five days and then weighing. This was found sufficient to dry the sample to constant weight.

CARBOHYDRATES.—The procedure was essentially as described by CLEMENTS (**12**). However, the term acid-hydrolyzable has been substituted for hemicellulose.

NITROGEN.—(*a*) *Total organic N including NH_4-nitrogen:* Dried material was used for these determinations and was subjected to the ordinary Kjeldahl method for nitrogen analysis.

(*b*) *Total soluble organic N including NH_4-nitrogen:* The autoclaved plant material was thoroughly ground in a handmill using generous quantities of water. The resulting material was then brought just to the boiling point at which time from 5 to 10 cc. of 10 per cent. acetic acid were added to the mixture to precipitate the colloidal material. The mass was then filtered, using suction. The residue was again diluted with water, brought to a boil, and then precipitated with acetic acid. This was done three times and the volume of the filtrate brought to 2 liters. One hundred cc. samples of this soluble nitrogen solution were placed in Kjeldahl flasks and digested according to the ordinary Kjeldahl method. The flames were kept low until most of the water had been driven off in order to prevent nitrogen loss. After digestion the NH_3 was distilled into standard H_2SO_4.

(*c*) *Amide nitrogen:* To 200 cc. of soluble nitrogen solution 12 cc. of conc. HCl were added and refluxed for three hours. To this, 50 cc. of a 10 per cent. MgO suspension were added in addition to boiling chips to prevent bumping, and paraffin to lessen foaming. This solution was then made alkaline with 15 cc. of saturated NaOH solution and distilled as for total soluble N. The amount of NH_4-nitrogen present was subtracted to give the amount of amide nitrogen. The amide was assumed to be present as asparagine, hence the results were doubled to take account of the amino group.

(*d*) *NH_4-nitrogen:* A 100-cc. sample of the soluble nitrogen extract was neutralized with 5 cc. of saturated NaOH solution and distilled. Paraffin was necessary to prevent foaming.

(*e*) *NO_3-nitrogen:* The material left from the ammonium distillation was diluted to approximately 200 cc. with distilled water. To this, 1 gm. of Devarda's alloy was added and distillation proceeded as before.

(*f*) *Diamino and basic nitrogen:* Five cc. of 20 per cent. phosphotungstic acid in a 2.5 per cent. H_2SO_4 solution were added to 100 cc. of soluble nitrogen extract which had been made 2.5 per cent. acid with H_2SO_4. The sample was then filtered and the Kjeldahl method used on the precipitate the same as for soluble nitrogen.

(*g*) *Alpha amino N:* This was determined with the Van Slyke apparatus.

CALCULATION.—For hourly changes in the leaves, the residual dry weight method was used. The material left behind after the removal of the carbohydrates represents the least fluctuating portion; hence absolute changes in amounts of different compounds can be calculated. The water basis was used for gradient calculations; the green weight for seasonal changes. Calculations based on total insoluble nitrogen were made but the significance of these was difficult to determine.

Discussion of data and conclusions

LEAVES

It must be remembered that the leaves were collected in the early morning at all times during the season, and that relatively low amounts of a given carbohydrate at this time of the day do not mean that it is not produced by the leaves in considerable quantities later in the day. For example, starch is produced in large quantities in both the upper and lower leaves (figs. 1, 2) during the day, while in the early morning it is nearly absent at all times (table I). Sucrose, likewise, follows the starch pattern of behavior. This suggests that the main function of starch and sucrose is one of temporary storage. Acid-hydrolyzable material fluctuated greatly during the day (figs. 1–3), yet in the morning the concentration was rather constant. This was especially true for the young leaves (table I), and indicates that this material is composed of substances having widely different functions. Judging from the action of starch, which may reach high concentrations during the day but be almost absent in the morning, it might be expected that the less permanent acid-hydrolyzable material is largely translocated at night, leaving the more permanent forms behind. Probably a part of this permanent material forms integral parts of the leaf protoplasm. Thus the seasonal changes in carbohydrates in the leaves must be studied with these as well as other facts in mind.

Early in the season sucrose was frequently absent from both young and old leaves at the time of collection (table I). Later in the season sucrose was always present in both, but not in large quantities. Figures 1 and 2 show that in hourly studies the young leaves have a greater average sucrose concentration during the day than the old leaves. The greater simple sugar values in the young leaves may be the cause of this. A slower rate of translocation due to a less developed phloem system accounts for the larger simple sugar values. Both the simple sugars and sucrose fluctuate more in the old than in the young leaves, suggesting further that the old leaves have the better conducting system.

Figure 1 shows that in the young leaves the simple sugar concentration reaches its maximum at 11 A.M., which corresponds to the maximum of solar radiation. This compares with HARTT'S work on the sugar cane (**24**). Fig-

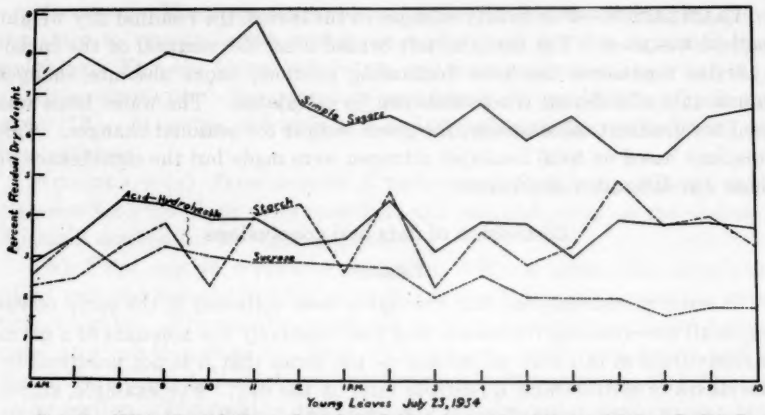

FIG. 1. Diurnal variation of carbohydrates in young leaves, 1934, calculated as percentage of residual dry weight.

ure 2 indicates that old leaves behave differently. Maximum simple sugar as well as total carbohydrate concentration comes at a time somewhat before the maximum of solar radiation and then drops slightly. With old leaves this drop may indicate the initiation of rapid translocation. There is then another increase, which may indicate that even the accelerated translocatory process cannot take care of the increasing amounts of photosynthetic products. After the maximum of solar radiation, simple sugars decreased in both old and young leaves with fluctuations until nightfall.

The hourly studies of the old leaves of 1933 (fig. 3) show that the ratio of simple sugars to other carbohydrates is much less than that of the old

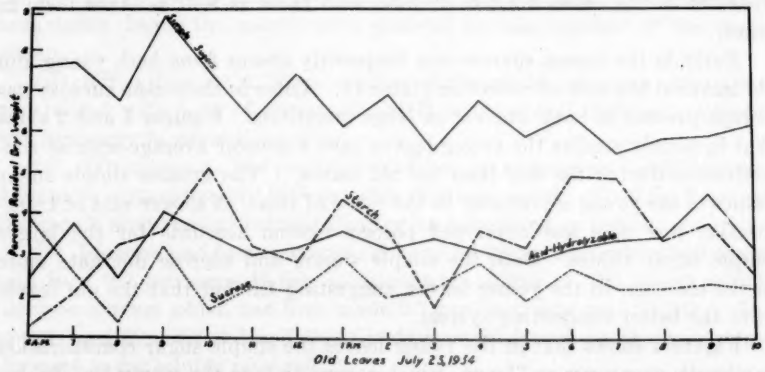

FIG. 2. Diurnal variation of carbohydrates in old leaves, 1934, calculated as percentage of residual dry weight.

leaves of the 1934 series. The concentration of the other carbohydrates was about the same. It is difficult to compare the results of the two series, however, because of the marked differences in weather conditions. The 1933 collections were made on a bright, still day of low humidity. The small hourly variations in starch and acid-hydrolyzable material suggest a more constant rate of translocation and synthesis for the 1933 series under unfavorable weather conditions. The intermittent cloudiness and the general haziness caused a low and fluctuating light intensity which possibly is reflected in the lower simple sugar concentrations. These did not reach their maximum until 6 P.M.

Comparing figures 2 and 3 with the work of CLEMENTS (**12**), it is interesting to note the decided differences in the relative proportions of the different carbohydrates. In his series, representing work done in Michigan, simple sugars were seldom the most abundant carbohydrate present. Figure 3 represents the closest approach of any of the series to the Michigan work,

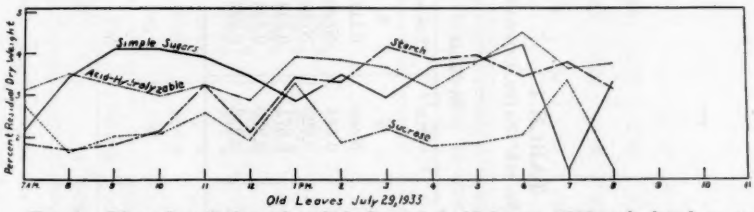

FIG. 3. Diurnal variation of carbohydrates of old leaves, 1933, calculated as percentage of residual dry weight.

but in this case the total carbohydrate concentration was considerably lower. Figure 2 shows a greater deviation still, the simple sugars being considerably more concentrated than any other carbohydrate, but in this case there was less difference in total carbohydrate concentration. Thus there are marked differences in the carbohydrate behavior of plants grown in Michigan and in Washington. To be sure, these differences are caused by the sum of the differences in light intensity, relative humidity, temperature, wind effects, length of day, and soil conditions.

It is interesting to compare the sugar concentrations in the young and old leaves during the day and early in the morning. The total sugar in the young leaves was somewhat higher during the day than in the old leaves (figs. 1, 2), but in the morning (table I) this condition was always reversed. This is thought to be due to the need of the young developing leaves for building material, thus reducing the sugar concentration to values which are lower than in the old leaves.

Simple sugars in the leaves increased in concentration in both young

TABLE I

SEASONAL CHANGES IN CARBOHYDRATES IN YOUNG AND OLD LEAVES, CALCULATED AS PERCENTAGE OF GREEN WEIGHT; 1934

TIME	STAGE	SIMPLE SUGARS		SUCROSE		STARCH		ACID-HYDROLYZABLE	
		OLD	YOUNG	OLD	YOUNG	OLD	YOUNG	OLD	YOUNG
		%	%	%	%	%	%	%	%
July 9	5 feet tall	0.387	0.573	None	0.117	0.112	0.132	0.487	0.823
July 16	6 feet tall	1.464	1.007	0.084	None	0.154	0.135	0.784	0.899
Aug. 3	Flowers opening	1.353	1.746	0.025	None	0.147	0.198	0.820	1.260
Aug. 13	Early seed stage	2.226	1.999	0.571	0.344	0.197	0.279	1.004	1.161
Aug. 21	Middle seed stage	1.920	1.627	0.320	0.304	0.125	0.152	0.748	1.063
Aug. 28	Mature seed stage	1.922	1.264	0.336	0.283	0.100	0.174	0.952	1.002

TABLE II

SEASONAL CHANGES IN NITROGENOUS SUBSTANCES IN OLD AND YOUNG LEAVES, CALCULATED AS PERCENTAGE OF GREEN WEIGHT; 1934

NITROGEN	JULY 9		JULY 16		AUG. 3		AUG. 13		AUG. 21		AUG. 28	
	OLD	YOUNG	OLD	YOUNG	OLD	YOUNG	OLD	YOUNG	OLD	YOUNG	OLD	YOUNG
	%	%	%	%	%	%	%	%	%	%	%	%
Total	0.401	0.630	0.357	0.516	0.362	0.407	0.368	0.404	0.360	0.464	0.384	0.524
Soluble	0.059	0.097	0.053	0.081	0.038	0.057	0.045	0.064	0.052	0.092	0.061	0.085
Basic	0.011	0.017	0.016	0.020	0.010	0.014	0.010	0.012	0.009	0.024	0.016	0.016
Amino	0.011	0.018	0.013	0.017	0.007	0.007	0.008	0.015	0.009	0.014	0.008	0.011
Amide	0.003	0.007	0.003	0.003	None	0.002	0.004	0.004	0.001	0.002	0.002	0.005
NH_4	0.003	0.005	0.004	0.004	0.004	0.004	0.004	0.005	0.005	0.012	0.005	0.006
NO_3	0.008	0.008	0.003	0.003	0.002	0.003	0.003	0.001	0.003	0.004	0.005	0.006

TABLE III

HOURLY CHANGES IN MOISTURE CONTENT OF YOUNG AND OLD LEAVES ON JULY 23, 1934, CALCULATED AS PERCENTAGE OF RESIDUAL GREEN WEIGHT

	6 A.M.	7	8	9	10	11	12	1 P.M.	2	3	4	5	6	7	8	9	10
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Young leaves	84.1	83.8	82.4	82.4	82.3	82.1	81.5	80.6	80.0	79.8	80.6	79.4	79.3	80.7	80.9	82.0	82.7
Old leaves	85.1	85.6	83.4	85.3	85.1	82.1	82.8	83.7	81.7	82.5	82.4	80.6	82.3	82.7	82.3	83.2	83.2

and old leaves until after seed formation. From this time on these decreased because of the great sugar utilization by the developing head.

The rôle of the leaves in synthesizing organic nitrogen is probably less important than their rôle in synthesizing carbohydrates: yet there are different amounts of nitrogenous material in the leaves at different times during the season, this amount being related to the need of the developing parts (table II). Soluble nitrogen decreases from early in the season until the head opens, exposing the ray flowers (August 3). The soluble nitrogen now increases in both young and old leaves. Total nitrogen follows the same pattern as soluble nitrogen, the changes being most marked in the young leaves. Soluble nitrogen follows a more or less inverse relation with the carbohydrates. After the early season high point, nitrate nitrogen is low until late in the season when high nitrate concentration in many parts of the plant probably causes a greater ingress into the leaves. This may likewise be due to a lowered reducase activity by the leaves. In the 1933 series, there was no such accumulation of nitrates in the leaves at the end of the season; this was related to its near absence in all parts of the plant for this season at this time. Neither the total nor the soluble organic nitrogen of the leaves dropped at the end of the 1934 series as they did in the 1933 series (table II).

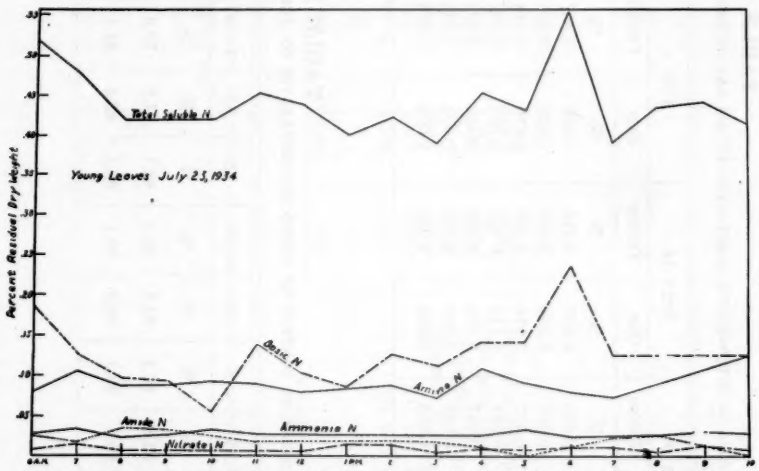

FIG. 4. Diurnal variations of soluble nitrogen in young leaves, 1934, calculated as percentage of residual dry weight.

This is associated with the presence of large amounts of nitrate nitrogen in the 1934 series, making destruction of the leaf proteins unnecessary for the developing seed. Basic nitrogen increased in the leaves as the plants approached maturity. The total nitrogen in the leaves per plant decreased at

the end of the season. This was due to the falling off of the dead lower leaves.

The hourly changes in the soluble nitrogen fractions probably show certain things regarding synthesis from inorganic nitrogen. In the case of the sunflower plant it is not known whether the leaves are very important in synthesizing organic nitrogen. Comparing figures 4 and 5, for young and old

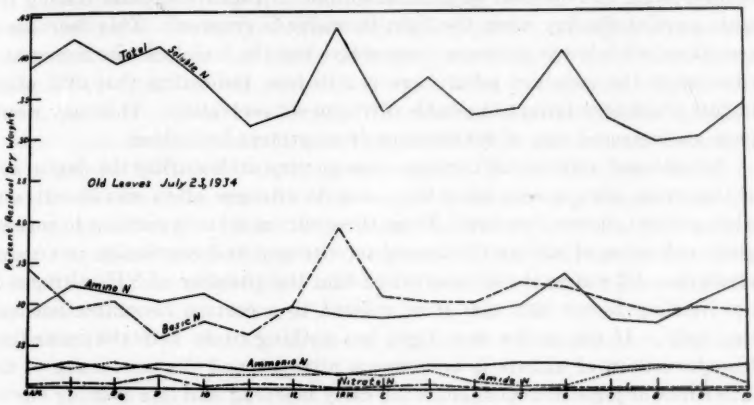

FIG. 5. Diurnal variations of soluble nitrogen in old leaves, 1934, calculated as percentage of residual dry weight.

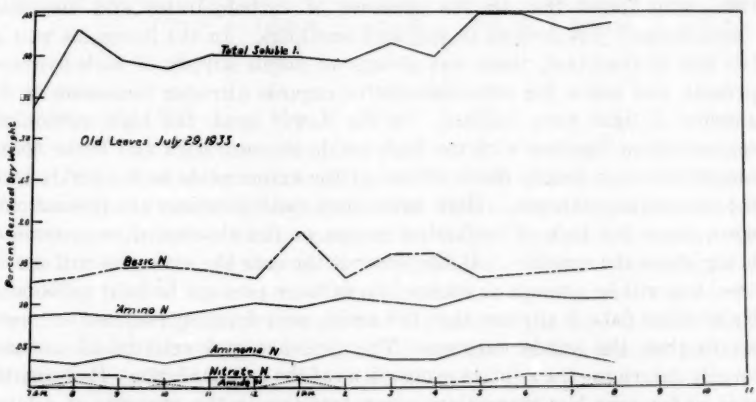

FIG. 6. Diurnal variations of soluble nitrogen in old leaves, 1933, calculated as percentage of residual dry weight.

leaves respectively, it can be seen that there are certain similarities as well as dissimilarities between them. The general trend in soluble nitrogen for

both series is to decrease from a high point in the morning, rise towards 11 A.M. with young leaves and 1 P.M. with old leaves, decrease in the afternoon, rise again at 6 P.M., fall off as night approaches, and finally the old leaves show evidence of again rising. The increase in late morning for the young leaves is slight, but considerable for the old leaves. The 6 P.M. increase was quite general, occurring in all three series at the same time. It is interesting to note that in general soluble nitrogen decreases during the main part of the day when the light intensity is greatest. This decrease is associated with lower moisture percentage, but the 6 o'clock rise comes at a time when the moisture percentage is still low, indicating that still other factors are determining the soluble nitrogen concentration. This may result from an increased rate of synthesis or from protein hydrolysis.

Nitrate and ammonium nitrogen change very little during the day in any of the series, always remaining low. Amide nitrogen often was absent, and when present, it was very low. From these curves there is nothing to suggest great reduction of nitrate to ammonium nitrogen and conversion to organic nitrogen. Of course it can be assumed that the quantity of NH_4 nitrogen is the limiting factor and that it is utilized to a certain concentration continuously. If this is the case, light has nothing to do with the immediate transformation of nitrate to ammonium nitrogen and the conversion of the latter into organic nitrogen, since the early morning and late evening curves show no appreciable differences from the curves of the rest of the day. These data lead to the same conclusions as were reached by PRIANISCHNIKOW (**35**), who found that in the presence of carbohydrates and ammonia, "asparagine" was formed in etiolated seedlings. In the leaves, as well as the rest of the plant, there was always an ample supply of carbohydrates present, and hence the requirements for organic nitrogen formation in the absence of light were fulfilled. In the flower head, the high ammonium concentration together with the high amide concentration and lower amino concentration indicates the synthesis of the amino acids to the amide from the ammonium nitrogen. High ammonium concentrations are present only when there is a lack of combining groups or the absence of an enzyme to bring about the reaction. If the latter is the case the ammonia will not be free, but will be present as ammonium salts or perhaps be held colloidally. From these data it appears that the amino acid forming enzymes are more active than the amide enzymes. The presence and activity of enzymes largely determine the relative proportions of the end products. It is possible that under even low ammonium concentrations, in the presence of a large amount of amide enzymes, amides only might be found present.

Amino and basic nitrogen are the important soluble nitrogen fractions analyzed for in the leaves. The "rest" nitrogen may be important, but its nature is unknown and hence it will not be discussed. Amino nitrogen

appears to be changed over into basic nitrogen at times. This is illustrated by the brief evening rise of basic nitrogen in the young leaves and the simultaneous drop in amino nitrogen (fig. 4). The inverse relation between these two nitrogen compounds may be seen in the evening rise for the old leaves (fig. 5). Another interesting general relationship appears to be that, in the morning and evening when the water percentage is greatest, the amino nitrogen rises while the basic nitrogen lowers. In the afternoon when the leaves become somewhat dehydrated, basic nitrogen appears to increase at the expense of amino nitrogen, especially in the young leaves. These, with other similar suggestions read from the graphs, suggest the interconversion of these two forms. The diurnal variations in the moisture content based on the residual green weight are not great, even on the windy day when the 1933 leaf collections were made. Table III shows the variations in the moisture content of young and old leaves calculated on the residual green weight basis. The actual differences between the residual green weight and green weight are not great. This is interesting, since data plotted on a green weight basis often have been skeptically received, for it has been assumed that moisture loss was great.

Table IV shows that the moisture content of leaves does not fluctuate

TABLE IV

MOISTURE CHANGES IN YOUNG AND OLD LEAVES FOR 1934 SEASON, CALCULATED AS PERCENTAGE OF GREEN WEIGHT

	JULY 9	JULY 16	AUG. 3	AUG. 13	AUG. 21	AUG. 28
	%	%	%	%	%	%
Young leaves ...	85.3	84.5	82.0	78.5	78.3	78.2
Old leaves	87.5	86.0	83.6	79.7	82.2	80.0

greatly after flower formation (August 3), both young and old leaves remaining fairly constant thereafter. The 1933 series which was extended over a longer period shows the same tendencies. The moisture content was always greater in the old than in the young leaves. In this connection it is interesting to note the constancy of the acid-hydrolyzable material in the young leaves through the greater part of the season. In the old leaves these substances were less constant. It is significant that during drought periods, the old leaves wilt and drop first.

BARK

In all bark divisions the starch content remained very low throughout the season. The acid-hydrolyzable material in the lower bark increased from 0.6 per cent. on a green weight basis early in the season to 1.4 per cent. at

the time of the opening of the head exposing the ray flowers. From this time on the concentration decreased. This indicates that the acid-hydrolyzable material is present as a temporary food and is drawn on by the head at the time of rapid growth during the maturation of the seeds. The concentration of acid-hydrolyzable material is smaller in the upper divisions of the bark, but the trend is the same.

The percentage of sucrose increased greatly in the lower and second bark regions (0.8 to 1.3 per cent. for the lower bark and 1.1 to 2.3 per cent. for the second bark) during the early seed development period (August 13), and then dropped off very rapidly, only to rise again. The same pattern is found in the upper bark, although it is less marked. Simple sugars and sucrose followed a more or less inverse pattern in all three bark divisions, thus indicating that there is some sort of an enzymatic equilibrium between the two forms which, in part, appears to be dependent upon the water concentration.

Total and soluble nitrogen in the bark sections fluctuated considerably during the season (table VI). The bark regions did not follow the same pattern in total nitrogen. The lower bark showed only a slight final accumulation of total nitrogen; the second bark showed considerable concentration but a final removal; and the upper bark showed a permanent accumulation. The total soluble nitrogen patterns were the same, all increasing at maturity. The nitrate nitrogen remained low until after the appearance of the ray flowers, at which time nitrates accumulate in all bark parts. Amino nitrogen was sometimes entirely absent from the bark as it was at the time of the appearance of the rays and at maturity in all bark parts. Amide nitrogen was always very low, often being absent in some bark sections, but usually not lacking in all bark sections at the same time. Basic nitrogen was the most abundant of the soluble nitrogen forms and gave the same type of pattern in all bark parts. Essentially, the basic nitrogen increases until the formation of the rays and then decreases through the early seed maturation, and finally accumulates at the end of the season. The final lowering in the percentage of simple sugars, sucrose, and other carbohydrates is probably in part connected with the increase of soluble nitrogen.

Flower head

The sunflower head is the chief "sink" in the plant late in the season after other parts have completed their development (table VII). Early in its growth, the upper stem uses the sugars as rapidly as they are translocated. At this time the sucrose concentration is zero, the simple sugars are present only in traces, and the acid-hydrolyzable material amounts to less than 0.4 per cent. of the green weight. From this point up to flowering time (August 3), acid-hydrolyzable material increases rapidly. The same was true for the

TABLE V

SUGAR CHANGES IN VARIOUS BARK SECTIONS DURING 1934 SEASON CALCULATED AS PERCENTAGE OF GREEN WEIGHT

BARK SECTION	JULY 9		JULY 16		AUG. 3		AUG. 13		AUG. 21		AUG. 28	
	SUCROSE	SIMPLE SUGARS	SUCROSE	SIMPLE SUGARS	SUCROSE	SIMPLE SUGARS	SUCROSE	SIMPLE SUGARS	SUCROSE	SIMPLE SUGARS	SUCROSE	SIMPLE SUGARS
	%	%	%	%	%	%	%	%	%	%	%	%
Lower	0.780	0.712	0.828	1.007	0.520	1.131	1.350	0.855	0.960	1.050	1.022	0.896
Second	.....	.....	1.115	0.529	0.581	1.714	2.340	1.350	1.488	0.624	1.260	1.050
Upper	.....	.....	.....	.....	0.534	1.453	1.044	1.557	0.693	1.151	1.104	0.816

TABLE VI

TOTAL AND TOTAL SOLUBLE NITROGEN CHANGES IN VARIOUS BARK SECTIONS DURING 1934 SEASON, CALCULATED AS PERCENTAGE OF GREEN WEIGHT

BARK SECTION	JULY 9		JULY 16		AUG. 3		AUG. 13		AUG. 21		AUG. 28	
	TOTAL	SOLUBLE	TOTAL	SOLUBLE	TOTAL	SOLUBLE	TOTAL	SOLUBLE	TOTAL	SOLUBLE	TOTAL	SOLUBLE
	%	%	%	%	%	%	%	%	%	%	%	%
Lower	0.149	0.034	0.124	0.020	0.081	0.026	0.087	0.019	0.082	0.019	0.090	0.029
Second	.....	.....	0.103	0.021	0.077	0.022	0.081	0.018	0.095	0.019	0.087	0.032
Upper	.....	.....	.....	.....	0.137	0.034	0.085	0.023	0.148	0.033	0.150	0.045

simple sugars and sucrose but these increased to the early seed development period (August 13). Starch was low at all times. In its early development, the seed had a very high concentration of sugars, up to 3.1 per cent. sucrose and 2.2 per cent. simple sugars, making a concentration of 5.3 per cent. (green weight basis). As the seeds mature the sugars are transformed into fat and acid-hydrolyzable material. There is a marked reduction in the sugar concentration at the end of the season in both the head and the seeds, but greatest in the seeds. CLEMENTS (**12**) determined the fat content of the entire head and found it to represent 18 per cent. of the total dry material. The part of the head other than the seeds represents a composite of tissue consisting of involucral bracts, bark, wood, pith, and flower parts; hence any concentration values of different organic compounds must be regarded with this in mind. In the head, the sucrose concentration is about one-quarter that of the seeds in the young stage, but keeps a fairly constant concentration; while the concentration in the seeds decreases, so that at the time of the last collection the differences in sucrose concentration were not great. The simple sugars drop in the rest of the head continuously, but still make up a concentration of 1.6 per cent. at the end of the season. In the head the acid-hydrolyzable material appears to function as a food reserve. In the early seed stage (August 13) this material was high, but dropped to a lower but fairly constant level toward maturity. The function of the head appears to be one of storage of food material from which the seeds may draw at a constant rate.

As the young flower bud develops, the total N/soluble N ratio increases very rapidly (table VIII). Interesting differences between the seeds and the rest of the head in the ratio of total N/soluble N exist. In the seed this ratio is large while in the head it is small. This indicates that the head is the supplying organ and the seeds the consuming one. The total nitrogen in the seeds increases very markedly. During the 1933 series, soluble N (green weight basis) decreased in the seeds until they approached maturity, at which time the concentration remained fairly constant. During the 1934 series there was little change in soluble nitrogen in the seeds (table VIII). However, seasonal changes in the seeds on a green weight basis must be critically viewed. As the seeds approached maturity, moisture percentage decreased rapidly and with this the total N/soluble N ratio increased, showing that there was a continuous transformation of soluble N into protein, as ECKERSON determined for wheat grains when they were dehydrated (**18**).

High ammonium, amino, and soluble nitrogen values for the head indicate either synthesis or storage of these forms in this tissue. The presence of a large amount of ammonium nitrogen especially favors the idea that synthesis of organic nitrogen is taking place in the head. The seeds initially possess large quantities of ammonium and amino nitrogen which decrease as

TABLE VII

CARBOHYDRATE CHANGES IN SUNFLOWER HEAD DURING 1934 SEASON, CALCULATED AS PERCENTAGE OF GREEN WEIGHT

CARBOHYDRATES	JULY 9	JULY 16	AUG. 3	AUG. 13		AUG. 21		AUG. 28	
	BUD	BUD	BUD	HEAD	SEED	HEAD	SEED	HEAD	SEED
	%	%	%	%	%	%	%	%	%
Acid-hydrolyzable	0.429	0.802	1.154	1.237	0.759	0.546	1.555	0.632	2.064
Starch	0.109	0.182	0.171	0.181	0.263	0.351	0.102	0.082	0.133
Sucrose	None	0.342	0.875	0.660	3.131	1.027	1.428	0.830	0.965
Simple sugars	0.133	0.588	1.185	2.640	2.185	2.041	1.300	1.661	0.233

TABLE VIII

NITROGEN CHANGES IN HEAD DURING 1934 SEASON, CALCULATED AS PERCENTAGE OF GREEN WEIGHT

NITROGEN	JULY 9	JULY 16	AUG. 3	AUG. 13		AUG. 21		AUG. 28	
	BUD	BUD	BUD	HEAD	SEED	HEAD	SEED	HEAD	SEED
	%	%	%	%	%	%	%	%	%
Total	0.247	0.284	0.336	0.241	0.251	0.144	0.479	0.174	0.959
Soluble	0.109	0.084	0.092	0.075	0.098	0.045	0.100	0.088	0.098
Basic	0.017	0.012	0.016	0.011	0.015	0.013	0.029	0.017	0.037
Amino	0.031	0.019	0.023	0.018	0.039	0.013	0.020	0.026	0.020
Amide	0.006	0.007	0.006	0.013	None	None	0.006	0.002	0.004
Ammonium	0.017	0.008	0.011	0.010	0.021	0.010	0.015	0.018	0.016
Nitrate	0.006	0.008	0.003	0.004	0.006	0.006	0.003	0.007	0.005

they mature, with the simultaneous increase in the basic nitrogen content, suggesting the conversion of the amino into the basic nitrogen.

The head seems to act as a storage as well as a synthesizing organ for organic nitrogen, and it is entirely possible that a considerable portion of the organic nitrogen formed after the flowering period is synthesized here. The young seeds contain large amounts of ammonium and amino nitrogen, indicating synthesis of organic nitrogen.

Roots

On the green weight basis the acid-hydrolyzable material of the root increases throughout its development but on a dry weight basis stays fairly uniform. It is very doubtful, however, whether the dry weight represents as true a picture as the green weight, even though the latter is unsatisfactory. Starch is always low.

Early in the season the simple sugar concentration is very low and sucrose is absent. Apparently the simple sugars are immediately used in the growth of the growing roots at this time, judging from the fact that there was no storage of sugars. The concentration of simple sugars increases somewhat as the root development slows down, but the sucrose increases much more rapidly and becomes the predominating sugar at the end of the season.

In the young roots (1934 season) amino nitrogen was high (0.011 to 0.013 per cent.). This decreased until after seed formation (0.001 to 0.002 per cent.) and then increased again at the end of the season (0.012 per cent.). The 1933 concentrations were low at all times, and the fluctuations not great. The total soluble nitrogen for both seasons followed the same pattern as did the amino during the 1934 season. Basic nitrogen increased throughout the season on a green weight basis but not on a dry weight basis. Total nitrogen remained fairly constant on a green weight basis but decreased on a dry weight basis.

The sunflower roots are not storage organs except under unusual conditions. Clements (**14**), working in Michigan, found that early in the 1927 season nitrates were stored in the roots up to 2 per cent. of their dry weight, decreasing to 0.05 per cent. at flowering, increasing again to 1.5 per cent. and finally dropping off to 0.2 per cent.

Wood

The wood contained small amounts of sugars early in the season. In the lower wood sucrose was entirely absent, the same as the roots, and the simple sugars low. From this point on the sucrose increased until flowering and then decreased. In the second and upper wood sections sucrose reaches its maximum in the early seed stage (August 13), then decreases, and finally is marked by a rise at the end of the season. The lower wood may be too far

TABLE IX

SUGAR CHANGES IN WOOD DURING 1934 SEASON, CALCULATED AS PERCENTAGE OF GREEN WEIGHT

Wood section	July 9		July 16		Aug. 3		Aug. 13		Aug. 21		Aug. 29	
	Sucrose	Simple sugars	Sucrose	Simple sugars	Sucrose	Simple sugars	Sucrose	Simple sugars	Sucrose	Simple sugars	Sucrose	Simple sugars
	%	%	%	%	%	%	%	%	%	%	%	%
Lower	None	0.030	0.098	0.019	1.634	0.138	1.500	0.210	1.305	0.464	0.457	0.305
Second	.....	.....	0.420	0.101	1.564	0.437	2.502	0.462	0.918	0.351	1.596	0.468
Upper	.....	.....	.....	.....	1.351	0.699	2.173	0.954	0.667	0.161	1.702	0.621

TABLE X

ACID-HYDROLYZABLE CHANGES IN WOOD DURING 1934 SEASON, CALCULATED AS PERCENTAGE OF GREEN WEIGHT

Wood section	July 9	July 16	Aug. 3	Aug. 13	Aug. 21	Aug. 28
	5 feet tall	6 feet tall	Flowering	Early seed stage	Mature seed stage	
	%	%	%	%	%	%
Lower	1.565	2.364	2.105	1.880	2.900	3.050
Second	.....	1.174	2.139	2.992	2.160	2.856
Upper	.....	.....	1.542	2.146	2.300	1.955

away from the sugar source of the leaves to feel the final flux of sugars as the seeds mature. Thus there is a large amount of sugar produced which is not used in growth or seed formation. Sucrose concentration is usually the greatest in the second wood and the lowest in the upper wood. Simple sugars are usually most abundant in the upper wood, although they are always much less concentrated than sucrose.

Acid-hydrolyzable material can be seen to be an important constituent of wood (table X). Although this material may act as food, the fluctuations shown in table X indicate that it is not drawn on to any great extent. Starch was found to be low at all times.

The changes in the nitrogen fractions indicate to some extent the functions of the wood in nitrogen metabolism. All of the wood sections decreased from rather high soluble nitrogen concentrations early in the season until the time of early seed formation. At this time the soluble nitrogen may increase. The amount of this increase is dependent partly on the amount of nitrate nitrogen present and its reduction to ammonia and partly on the degradation of protein. The most marked increase of soluble nitrogen was associated with the greatest decrease in total nitrogen. The second wood shows little change in either total or soluble nitrogen. The third wood region is marked by a decrease in both total and soluble nitrogen. This can be explained by assuming that this wood is closer to the "sink" and hence the soluble nitrogen formed from protein degradation migrates away more easily and rapidly than in the other wood regions.

Amino nitrogen fluctuates more than the other organic nitrogen forms. It is initially high in all wood parts and then decreases, rising again in the early seed formation and dropping off to zero in all wood parts at the end of the season. Nitrate nitrogen accumulated in all parts late in the season, but was not present in large amounts before this time. This contrasts with the 1933 series in which nitrate nitrogen was low at all times.

Pith

Table XI shows that in the early season all parts of the pith contain little or no simple sugars. It must be remembered that the region which comprises the second pith is later divided into the third and upper pith sections. In the second pith the simple sugars are high at the time of the appearance of the ray flowers and then drop, only to rise again as the plants approach maturity. Sucrose is usually much less concentrated than the simple sugars. The third and upper pith follow the same pattern, but the concentration in the upper is much greater than in the third which in turn is much greater than in the second. In other words, the simple sugar concentration drops off very rapidly in the pith from the top of the plant to the bottom. At the time of the high simple sugar concentration, sucrose is comparatively

TABLE XI

SUGAR CHANGES IN PITH DURING 1934 SEASON, CALCULATED AS PERCENTAGE OF GREEN WEIGHT

PITH SECTION	JULY 9		JULY 16		AUG. 3		AUG. 13		AUG. 21		AUG. 28	
	SUCROSE	SIMPLE SUGARS	SUCROSE	SIMPLE SUGARS	SUCROSE	SIMPLE SUGARS	SUCROSE	SIMPLE SUGARS	SUCROSE	SIMPLE SUGARS	SUCROSE	SIMPLE SUGARS
	%	%	%	%	%	%	%	%	%	%	%	%
Lower	None	None	None	None	0.039	0.061	None	None	None	None	0.096	0.073
Second	None	Trace	Trace	None	0.211	0.507	0.087	0.045	0.067	0.027	0.336	0.159
Third	.....	.....	.....	.....	0.384	1.680	0.422	0.547	0.162	0.260	0.103	0.063
Upper	.....	.....	.....	.....	.....	.....	0.520	3.068	0.513	1.061	0.427	0.855

TABLE XII

NITRATE AND AMMONIUM NITROGEN CHANGES IN PITH DURING 1934 SEASON, CALCULATED AS PERCENTAGE OF GREEN WEIGHT

PITH SECTION	JULY 9		JULY 16		AUG. 3		AUG. 13		AUG. 21		AUG. 28	
	NH_4N	NO_3N	NH_4N	NO_3N	NH_4N	NO_3N	NH_4N	NO_3N	NH_4N	NO_3N	NH_4N	NO_3N
	%	%	%	%	%	%	%	%	%	%	%	%
Lower	0.005	0.050	0.003	0.018	0.004	0.015	0.003	0.019	0.003	0.057	0.001	0.053
Second	0.021	0.030	0.006	0.027	0.004	0.012	0.004	0.010	0.002	0.088	0.006	0.080
Third	.....	.....	0.009	0.006	0.014	0.003	0.006	0.004	0.006	0.042	0.027	0.052
Upper	.....	.....	.....	.....	.....	.....	0.011	0.003	0.013	0.025	0.025	0.014

TABLE XIII

AMINO AND TOTAL SOLUBLE ORGANIC NITROGEN CHANGES IN PITH DURING 1934 SEASON, CALCULATED AS PERCENTAGE OF GREEN WEIGHT

PITH SECTION	JULY 9		JULY 16		AUG. 3		AUG. 13		AUG. 21		AUG. 28	
	SOLUBLE	AMINO	SOLUBLE	AMINO	SOLUBLE	AMINO	SOLUBLE	AMINO	SOLUBLE	AMINO	SOLUBLE	AMINO
	%	%	%	%	%	%	%	%	%	%	%	%
Lower	0.024	0.010	0.022	0.019	0.026	None	0.024	0.002	0.017	0.005	0.028	None
Second	0.077	0.036	0.032	0.008	0.028	0.001	0.024	0.005	0.016	0.002	0.024	0.008
Third	.....	.....	0.059	0.014	0.060	0.017	0.028	0.005	0.034	0.009	0.088	0.040
Upper	.....	.....	.....	.....	.....	.....	0.059	0.012	0.061	0.026	0.114	0.045

insignificant, but as the hexose concentration decreases, it approaches the sucrose concentration. This is an interesting contrast with the wood. The wood stores sucrose predominantly, while in the main the pith accumulates simple sugars. Why the differences? SPOEHR (**38**), working with the cactus, concluded that high water concentration and lower temperatures were associated with a decrease in the polysaccharides and an increase in the monosaccharides, while a lower water concentration and higher temperatures were associated with an increase in the polysaccharides and a decrease in the monosaccharides. The temperature relationships cannot be considered here since no tissue temperature measurements were made. However, the water relationships were marked. The pith contains from 90 to 97 per cent. moisture, and the wood from 65 to 85 per cent. moisture. Thus the differences are associated with differences in the water percentages of the tissues. The data of BISSON and JONES (**5**) on the pea show the same relationship. Sucrose was considerably higher in the shelled peas than in the pods, while the simple sugars were present in the reverse order. These data are associated with a greater moisture percentage of the pods than was present in the peas.

Acid-hydrolyzable material is low in all pith parts and fluctuates but slightly through the entire season. Starch is low at all times.

Total nitrogen in the pith in the different parts does not fluctuate greatly, but the different parts do not follow the same pattern. Soluble nitrogen is likewise variable in its pattern behavior. The soluble nitrogen of the third and fourth pith sections increases very markedly, while in the first and second pith the soluble nitrogen increase is small.

Nitrate nitrogen is stored predominantly in the pith (table XII) and probably in the rays. This happens only under conditions of excessive nitrate absorption. MOLISCH (**30**) noticed the storage or accumulation of nitrates in the pith of sunflower plants when they were grown upon very fertile soils. The 1933 series showed no nitrate accumulation in the pith, while the 1934 series showed high accumulations at times. Early in the season nitrates accumulated and then decreased through flower formation and early seed development. As the seeds approached maturity, the nitrate nitrogen again accumulated in the pith, up to 0.08 per cent. in the second pith.

In general ammonium and soluble nitrogen increase progressively from the lower to the upper pith. Ammonia may reach very high levels in the upper pith. In general increases in amino nitrogen correspond with increases in ammonium nitrogen. Amide nitrogen is usually very low, even in the pith, although early in the season it is fairly concentrated. Basic nitrogen shows considerably less fluctuation than any of the other organic nitrogen forms for which analyses were made. This probably indicates that

it is less important as a translocatory form than either amino acids or amides. From the presence of ammonia and amino nitrogen apparently the upper pith is an important synthesizing tissue for organic and ammonium nitrogen. Likewise the frequent high amino/soluble organic nitrogen ratios support this conclusion (table XVI). It should be remembered that the pith cells become surrounded by air as the plant approaches maturity. Since the insoluble organic nitrogen in the pith is always low and its changes not always associated with increases in amino and ammonium nitrogen, it seems unlikely that much of the increase in ammonium and amino nitrogen results from proteolysis.

From the data which have been presented, the upper pith, flower head, and seeds appear to be regions of organic nitrogen synthesis. The bark, wood parenchyma, and leaves likewise appear to be active in this respect. Leaves are not thought to be so important in the synthesis of organic nitrogen as has been generally assumed.

Translocation

Carbohydrate movement

Sucrose was at all times less concentrated in the leaves than in the bark. Mason and Maskell (**28**) observed the same for cotton plants. Table XIV illustrates the type of gradients observed in sucrose and simple sugars. Usually simple sugars are more concentrated in the leaves than in the bark, so there is a conversion of these into sucrose between the leaves and the bark or in the bark. Whether the bulk of this conversion takes place in the leaves or the bark probably depends on the time of day. Early in the morning sucrose was low or entirely absent in the leaves, indicating that if there was any conversion of the simple sugars into sucrose, it occurred in the bark. During the day, however, sucrose became fairly concentrated in the blades, indicating a conversion of simple sugars into sucrose there.

The gradients in the bark show that from the second bark to the upper bark there is a decrease in sucrose and an increase in simple sugars. The "bark and wood" section has a higher sucrose value than the upper bark, but it is probably a result of high sucrose value in the wood (table IX). From the second bark to the first sucrose decreases. During the period of early seed formation the head has a higher simple sugar concentration than the bark, but is lower in sucrose. At this time, also, the sucrose concentration of the seed is considerably greater than for the rest of the head. Here it must be remembered that the head is a large mass of tissues, parts of which are high in one sugar form and parts in another. However, it seems doubtful that there is a continual decrease of sucrose in the sieve tubes from the head into the seeds. On the contrary, the association of increased simple sugar sucrose ratios with increased percentage in tissues suggests that the

invertase equilibrium point is affected by different percentages of moisture. HARTT (**24**) noticed that a high moisture percentage brought about by rain favored translocation in the sugar cane in the form of reducing sugars.

In translocatory tissue, it has been shown that both sucrose and the reducing sugars are present. The simple sugar/sucrose ratio has been shown to be associated in part with moisture percentage. Because of the apparent sensitivity of invertase to changes in moisture percentage, temperature, pH, etc., it is not surprising that opinions have varied regarding the form in which sugars move. When sucrose is the dominant sugar in translocatory tissue, it would seem as though this is the more important moving form, and if the simple sugars are the most abundant they probably are the more important moving forms. In still other cases these sugars may move together in nearly equal proportions. It is possible that little actual movement takes place except near the surfaces of colloids (**13**), and it is likewise possible that movement takes place on a gradient basis on the surface of colloids.

NITROGEN MOVEMENT

In the 1934 series, the bark gradients were various, but always followed a definite pattern. Table XV may be referred to as typical of the nitrogen data. The percentage of total and soluble nitrogen was always higher in the lower bark than in the second bark. Expressed on a water, dry, or green weight basis, the gradients from the second bark to the head were always negative. The percentages followed this scheme: bark 1 > bark 2 < bark 3 < bark and wood < head < seed. (Table XV.) The nitrate gradients in the bark were various, sometimes being positive and sometimes negative. Nitrates enter the roots and pass rapidly out into the lower stem sections, but under high nitrate conditions, nitrates may accumulate in the roots. Once in the lower stem region the nitrates scatter throughout the tissues, the excess being concentrated predominantly in the pith. In the bark, nitrates are reduced. Ammonium and amino nitrogen increase progressively up the stem, the ammonium nitrogen being the first to decrease in amount (in the bark). Thus it appears that the increased concentrations of the amino nitrogen result from a rapid ammonium utilization. Thus the data appear to show that the gradients are a result of nitrate reduction to ammonia and then the formation of amino acids, and hence it is likely that the negative gradients are of no significance in translocation.

Leaf fluctuations during the day indicate one of two things. Either these fluctuations result from different rates of synthesis and movement of organic nitrogen or from conditions affecting the equilibrium between soluble organic nitrogen and protein. The small amounts of nitrates and ammonia in the leaves, as is shown in the hourly studies, indicate a rapid utilization of these forms. However, it is impossible to say just what portion of the

organic nitrogen is synthesized in the leaves. The soluble nitrogen gradients out of the leaves (table XV) into the bark are positive. This contrasts with the negative gradients obtained by MASKELL and MASON for the cotton plant (**27**).

In the pith the ammonium gradients up the stem are essentially positive at all times, but at the end of the season (compare tables XII and XIII) the ammonium concentration is higher in the third than in the upper pith. At this time the amino concentration is lower in the third than it is in the upper pith, thus indicating that the disappearance of the ammonia is due to the formation of amino acids.

In the pith practically at all times high ammonium concentrations are associated with a very high amino content. At these times the ratio of amino nitrogen to total soluble organic nitrogen (not including ammonia) is greatest (table XVI). Early in the season in the lower pith the amino/soluble organic nitrogen ratio is high, and this is associated with a comparatively low ammonium concentration (compare tables XII and XVI). This indicates that the lower pith at this time has a sensitive mechanism for the

TABLE XIV

TYPICAL GRADIENT OF SUGARS FROM LOWER BARK TO SEEDS, AND YOUNG AND OLD LEAVES FOR COMPARISON, CALCULATED AS PERCENTAGE OF WATER WEIGHT; AUGUST 13, 1934

	LOWER BARK	SECOND BARK	UPPER BARK	BARK AND WOOD	HEAD	SEED	YOUNG LEAVES	OLD LEAVES
	%	%	%	%	%	%	%	%
Sucrose	1.59	2.85	1.21	1.74	0.80	3.71	0.44	0.71
Simple sugars	1.01	1.65	1.89	3.03	3.16	0.80	2.55	2.77

synthesis of amino acids, but a rather poor reduction mechanism since there are large amounts of nitrates present. The greater ammonium nitrogen concentration of the upper pith at this time does not necessarily indicate a weak synthesis of amino acids, for it may be that greater reducase activity furnishes more ammonia than can be used. One of the limiting factors is the complete absence of sugar in the pith at this time, indicating that the carbohydrate is being used as rapidly as it is received. As the season progressed, the amino/soluble organic nitrogen ratio decreased in all parts and the ammonium trend was generally the same. If the amino acids are the chief assimilatory and translocatory products, it would appear that the decrease is brought about by a rapid utilization of amino acid by the developing parts, and thus they are drawn upon to a greater degree than any of the other organic nitrogen forms. The appearance of large amounts of

TABLE XV

TYPICAL GRADIENT OF SOLUBLE NITROGEN FRACTIONS FROM LOWER BARK TO SEEDS, AND YOUNG AND OLD LEAVES FOR COMPARISON, CALCULATED AS PERCENTAGE OF WATER WEIGHT; AUGUST 13, 1934

NITROGEN	LOWER BARK	SECOND BARK	UPPER BARK	BARK AND WOOD	HEAD	SEED	YOUNG LEAVES	OLD LEAVES
	%	%	%	%	%	%	%	%
Soluble	0.0221	0.0214	0.0275	0.0537	0.0896	0.1165	0.0813	0.0561
Basic ...	0.0112	0.0078	0.0073	0.0061	0.0137	0.0172	0.0155	0.0120
Amino ...	0.0039	0.0039	0.0053	0.0150	0.0211	0.0458	0.0192	0.0104
Amide ...	0.0003	None	0.0052	Trace	0.0154	None	0.0049	0.0055
NH_4	0.0029	0.0038	0.0025	0.0070	0.0115	0.0241	0.0067	0.0050
NO_3	0.0061	0.0039	0.0067	0.0060	0.0048	0.0075	0.0033	0.0015

sugar in especially the upper pith removes the possibility of sugar being the limiting factor, as it may have been at the start of the season. Because of this, it is likely that the formation of amino acids is speeded up to the point where some other limiting factor comes into play. The final accumulation of amino acids and ammonia in the pith appears to be caused by a decreased consumption of the amino acids because of the slowing down of growth as the seeds approached maturity. The increased concentrations of ammonium nitrogen again, as at start of the season, can be associated with lower sugar concentrations. This in part accounts for the ammonium concentration in the third pith being higher than in the upper at the end of the season, since (table XI) the sugar was much less concentrated in the third than in the upper pith. All this tends to point toward the importance of the pith in synthesizing amino acids and the importance of the amino acids as translocatory and building material.

After the formation of the head and just before and during the enormous increase in its size, in which the nitrogen content more than doubled itself each week for three weeks, ammonium compounds reach very high values in

TABLE XVI

AMINO/SOLUBLE ORGANIC NITROGEN RATIOS IN VARIOUS PITH PARTS FOR 1934 SEASON

PITH	JULY 9	JULY 16	AUG. 3	AUG. 13	AUG. 21	AUG. 28
Lower	0.554	0.596	0	0.085	0.326	0
Second ...	0.657†	0.308	0.062	0.262	0.118	0.032
Third	.....	0.391	0.378*	0.213	0.360	0.660†
Upper	.....	.....	.....	0.259*	0.546*	0.500†

* Between 0.01 and 0.02% NH_4 nitrogen.

† Between 0.02 and 0.03% NH_4 nitrogen.

the head and seeds. It is possible that a part of this ammonium nitrogen resulted from amide hydrolysis (1). In the wood, the smallest amount of nitrates may be in the lower wood, while with each successive wood section the amount of nitrates may increase. This probably represents gradients of the nitrates which are stored in the medullary ray tissue, as would be expected from the behavior of the pith. Hence this does not necessarily represent tracheae and tracheid gradients.

The soluble nitrogen gradient was decidedly negative up the stem. How then, if nitrogen moves on a gradient plan, can the organic nitrogen manufactured down the stem ever reach the top? Basic nitrogen forms marked positive gradients up the stem into the head, but a decided negative gradient into the seeds. The high concentration of this form of nitrogen in the seeds suggests that basic nitrogen is more important as an intermediate building block in the formation of proteins than it is in translocation.

At times amide nitrogen may be very low in the lower bark and increase in each successive bark section, while the amino nitrogen may be in the reverse order. Sometimes amide is absent from certain bark sections, while at other times amino nitrogen is absent. The gradients of ammonium nitrogen are various, sometimes being almost absent from certain bark sections. The nitrate patterns are likewise indefinite in the bark. There apparently is a constant reduction of the nitrates in this tissue and a constant movement of nitrates from the wood. From this it would seem as though the chief form of nitrogen movement may shift from time to time, sometimes being nitrate, sometimes ammonia, sometimes amino, other times amide (not often because of its usual low concentration or absence), and in still others all four may take part.

Summary

1. The sunflower plant was subjected to diurnal as well as seasonal studies of its organic nutrition, organic solute translocation, and tissue functions. The organic materials studied were the carbohydrate and nitrogen fractions as well as the moisture relations.

2. The sensitivity of young leaves to carbohydrate fluctuations is not much greater than for old leaves. High reducing sugar values in the young leaves do not result in much higher sucrose values.

3. There were marked differences between the carbohydrate proportions found in leaves in the sunflowers grown in Washington and those grown in Michigan. In both the young and old leaves in the 1934 series, the simple sugars accounted for from one third to one half the carbohydrates.

4. The high simple sugar concentrations and fluctuations in the leaves correlated with much smaller sucrose concentrations, favoring the prevailing idea that the simple sugars are the first products of photosynthesis.

5. Positive gradients of sucrose were obtained for the bark, but these are

believed to be associated more with the water percentage of these tissues rather than indicating that this sugar is moving from "source" to "sink" on a gradient plan. The gradient of sucrose from the head into the seed is negative. This again is associated with the percentage of moisture in the respective tissues.

6. The wood stores sucrose predominantly, while the pith stores simple sugars. This is correlated with the moisture percentages of these tissues.

7. Simple sugars reach their maximum during the time of the opening of the head, exposing of the ray flowers, and for a short time thereafter. This is true for almost all of the tissues of the plant.

8. The pith is active as a storage tissue and as a tissue synthesizing organic nitrogen of which the amino acids are the most important. The pith is essentially void of sugars until the time of flowering and of early seed development. Nitrates may be stored in the pith either early or late in the season. During the period of rapid growth of the head, the nitrates stored up early in the season decrease.

9. In all parts of the sunflower plant starch is almost absent, except in the leaves during the day. At this time it, together with the acid-hydrolyzable material, performs the function of removing from solution a part of the excess sugar which was not immediately translocated.

10. The acid-hydrolyzable material which appears as a temporary storage form is nearly all translocated from the leaves at night. This is shown, especially in the young leaves, by the fairly constant percentage of this material in seasonal leaf collections, and this correlated with its large fluctuations during the day.

11. The diurnal fluctuations of soluble organic nitrogen in the leaves suggest that synthesis may take place here. The low concentrations of ammonium and nitrate nitrogen suggest a rapid conversion of these forms into the organic nitrogen.

12. The data at least strongly suggest that some of the organic nitrogen is synthesized in parts other than the leaves. The most active regions in this respect appear to be the upper pith and the head.

13. Gradients of soluble nitrogen out of the leaves into the bark are always positive. However, gradients of total soluble, amino, and ammonium nitrogen are nearly always negative up the stem. This is true for the bark, wood, and pith. It is possible that some of the gradients which appear negative on a gross macrochemical basis are actually positive on the surface of colloids (**13**).

14. Amides are never very abundant and are often absent. Ammonium nitrogen is probably present as ammonium salts or held to the surfaces of colloids.

15. As the seeds mature, large amounts of unused organic food material

may collect in the different plant parts, this collection being most noticeable in the upper regions.

16. The hourly fluctuations of moisture in the leaf blades, when calculated on a residual green weight basis, are not great. The moisture content of the older leaves was always greater than that of the young leaves.

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FURTHER STUDIES ON EXUDATION IN CUCURBITS

ALDEN S. CRAFTS

Introduction

With DIXON's recognition (7) that the movement of organic materials may take place through the phloem at rates sufficient to explain normal translocation, few advocates of the xylem theory remain, and attention is again centered upon the sieve tube hypothesis. The osmotic mechanism proposed by MÜNCH (14) to explain mass flow through sieve tubes is accepted by DIXON (7) and others (9). To some it may seem that the long search for a satisfactory explanation of the movement of organic materials in the plant is nearing an end.

There appears, however, to be a rational objection to the sieve tube theory. As early as 1835, VON MOHL (13) questioned the perforation of the sieve plate. KUHLA (10) and SCHMIDT (16) have cast further doubt on its structure. Faced by SCHMIDT's work on the one hand and by RUHLAND'S (15) statement as to the impermeability of sieve tubes on the other, and feeling that I had substantiated the studies of both these men, I proposed in 1931 (2) that a mass flow of nutrients in solution might pass through the intermolecular spaces in the highly hydrated phloem walls.

My use of the Poiseuille formula for flow through capillaries indicated that resistance to such flow would be high. It seemed possible, however, that if the formula were altered to apply to flow between parallel plates, then the second power function of r, the radius of the capillary, as given in my modification of this relation (2, p. 14), might be replaced by a first-power function of d, the distance between the plates (4, p. 213). This was not the case; and, since careful consideration of the structure of cellulose as determined by x-ray analysis gave no hope for intermolecular spaces wide enough to accommodate mass flow under the existing gradients, this theory was abandoned (3, 4, 5).

In studying the mechanism by which assimilated materials enter the phloem and exit from it, I needed more information on the detailed structure of sieve tubes. Most of the descriptions in the literature were of mature elements, and those giving more complete details did not present a consistent picture of sieve tube ontogeny.

In studies on the sieve tubes of cucurbits (4), I attempted to describe the various stages in the development of these elements and soon realized that my early experiments checking RUHLAND'S work told only part of the story. By using a variety of methods, I studied not only the form and contents of the elements at different ages but also their osmotic properties, reactions to vital stains, protoplasmic properties, and general structural

relations. These studies on the sieve tubes of cucurbits (**4**), potato (**5**), and two *Nicotiana* species (**6**) have been described. The outstanding conclusion of this work was that, although the young sieve tube element has the properties of a normal living cell, with maturity it goes through a series of unique changes resulting in a cessation of protoplasmic streaming, a disappearance of all inclusions except sieve tube plastids, and, so far as could be determined from the few species studied, a loss of the property of semipermeability.

The implications of this finding are apparent. With the sieve tubes completely permeable at maturity, their vacuoles may constitute the principal channels for movement. Mass flow, which rates and gradients (**1, 2, 4, 5, 12, 14**) would seem to demand, could occur with only the end walls to offer resistance. One radical departure from the classic sieve tube theory as elaborated by MÜNCH, however, would be required. With the possibility of a certain amount of movement through the walls, a "limiting layer," as proposed in my earlier publication (**2**), would be required.

The weakness of my theory of mass flow through walls was obvious and was pointed out by many of my colleagues. The essence of these criticisms has been well presented by STEWARD and PRIESTLEY (**17**). At present there seems to be no doubt that the resistance to movement through the walls over the total distance traversed would be too great. I should like to point out in this connection that my "rejection of the sieve tubes as the path for translocation" (**17**) rested *principally* upon the data of SCHMIDT (**16**) and RUHLAND (**15**), and that choice of the walls *followed* a search for some other channel. Only after the sieve tubes appeared to be eliminated were the large cross-sectional area and the high hydration of phloem walls recognized and the theory proposed that these walls might constitute the actual conduits.

STEWARD and PRIESTLEY, going further, have questioned my use of certain data on exudation from cucurbits. In their words, "It seems much more probable that in a succulent plant of the type used the exudation from the phloem is not an indication of a normally occurring mass flow at all, either in the walls or sieve-tubes, but may be due rather to the turgor of surrounding tissues and the release of tissue tensions consequent upon cutting. A new cut then merely opens up a new length of stem." They question further, and with some justification, my comparison of "the rate . . . observed over a period not exceeding five minutes . . . with the rate calculated as necessary to account for uptake of dry matter by a pumpkin . . . assumed uniform over a period of 100 days." These questions are raised in the face of data (**2**, pp. 18, 19) indicating that exudation may occur at rates equivalent to flow of over 2 cm. per minute through the *total phloem* while the section removed each minute need not exceed 1 mm. in thickness;

and the statement that "a volume of solution equal to the *volume* of the *total phloem* in 13.7 cm. of stem flowed out during the 21 minutes, about 1 cm. of stem having been removed during the collection (**2**, collection C, table VII, p. 19).

The present paper concerns primarily this matter of the relation of phloem exudation in the cucurbit to normal movement of assimilated foods. It indicates that more than cell turgor and tissue tensions are involved in the exudation process, and that the flow, whether "normal" or not, may move sufficient food material over great enough distances to account for observed rates of growth and storage.

Experimentation

Since my previous work with cucurbits (**4**), several perplexing questions have arisen concerning exudation. Having an opportunity to study some squash plants growing in culture solutions in the greenhouses of the Plant Nutrition Division at Berkeley, I performed a number of experiments that should clarify some of these questions.

The first problem was to distinguish between xylem and phloem exudation and to find the conditions required for separating the two. This distinction proved rather simple with these plants. As they were growing in aerated full nutrient solution and were developing rapidly, root absorption was very active, and to collect xylem sap it was necessary only to cut the plant below the lowest leaf. As soon as the water deficit caused by transpiration was satisfied (within 5 minutes or less in these experiments), xylem sap flowed from the cut stump, and exudation continued for several days until food reserves in the root became exhausted. If it was desirable to obtain the xylem exudate free of contamination from the phloem, steaming of the hypocotylar region would coagulate the phloem sap *in situ;* and, after a few minutes had been allowed for washing the cut surface clean, xylem sap could be collected in a relatively pure form.

To obtain pure phloem exudate the excised portion of the same plant could be used. If a 1 mm. slice was cut from the internode every two minutes, phloem exudate could be collected over a period of half an hour or more. Since this excised shoot might be 8 feet or more in length, an appreciable volume of sap could be collected. It continued to flow long after the leaves had wilted, and a positive phloem pressure could be detected after the whole stem had become flaccid. It seems highly doubtful that this flow could be attributed either to cell turgor or to tissue tension.

For extended collections of phloem exudate, the stem had to be maintained in a more natural condition; another method, accordingly, was used. Transpiration from the leaves maintained a subatmospheric pressure in the xylem of these plants throughout the day and during most of the night.

If the initial cut was made at the tip of the shoot, therefore, pure phloem exudate could be collected with little effect upon the normal functioning of the plants.

Examination of the xylem pressure conditions, either by dissection under the binocular or by cutting under eosin solution, proved that at the tips of these plants xylem pressure was subatmospheric from early morning until well after sundown. Water was being lost at a higher rate than it was being absorbed, although root pressure could readily be demonstrated by removal of the transpiring surface.

The next problem concerned the duration of phloem exudation from healthy plants. Previous tests had shown that it could be maintained over a period of 4 hours (**4**). The first run in the present experiments covered a 12 hour period. At 9:34 A. M. on July 10, four plants were selected and collections started. Two of the plants were in aerated and two in non-aerated solutions. For the collections small glass test tubes, $\frac{3}{16}$ inch in bore and 3 inches long, were used, a tube being slipped over the end of the stem as soon as it was cut. In these collections a slice of stem about 1 mm. in length was removed every 2 minutes. Each time, before the cutting, the free sap adhering to the end of the stem was wiped off and was teased down the side of the tube with a small dissecting needle. The stems were placed in a sloping position so that the sap flowed downward and remained in the bottoms of the tubes. The four plants were handled simultaneously until 1:40 P. M., when exudation from the unaerated plants became so slow that collection was impracticable. The collections on the two plants in aerated culture solutions were then made every minute and continued until 10:00 P. M. During the collection several internodes were removed, and each node with a portion of the two accompanying internodes was saved. These nodes were kept in cold tap water and were sectioned on the following day for phloem measurements. The data from the collections are presented in table I.

Obviously the plants in unaerated culture solution suffered from slow water absorption by the roots. By noon they had both wilted, and phloem exudation had dropped to less than half of that of the other two plants. The latter maintained a fair rate throughout the afternoon until about 8:30 P. M., when, with a rapid drop in water loss, the rate increased appreciably. By 10:00 P. M. the water deficit had been completely satisfied, and xylem exudation had started. The collections were stopped, and a test tube was placed under the end of each stem. By morning there were about 10 cc. in each test tube. This was a watery solution of low concentration and, since the ends were not cut after 10:00 P. M., consisted principally of xylem sap.

According to these data (table I), exudation from the phloem may be

TABLE I

PHLOEM EXUDATION FROM SQUASH. VOLUME AND RATE OF FLOW FROM CUT STEMS. COLLECTIONS MADE JULY 10, 1934

PLANT	COLLECTION		WEIGHT OF SAP		DRY WT. AS PERCENTAGE OF FRESH	PHLOEM AREA	LINEAR* RATE OF FLOW PER HR.	TEMPERATURE OF GREENHOUSE
	No.	TIME	FRESH	DRY				
			gm.	*gm.*	%	*cm.*²	*cm.*	°*C.*
A Not aerated	1	9:34–10:36	0.302	0.019	6.4	0.007	43.1	26.0
	2	10:36–11:39	0.167	0.008	4.7	0.015	11.1	27.5
	3	11:39–12:38	0.083	0.004	4.8	0.019	4.4	29.2
	4	12:38– 1:40	0.041	0.002	4.6	0.019	2.2	29.2
B Not aerated	1	9:38–10:37	0.156	0.013	8.1	0.010	15.6	26.0
	2	10:37–11:40	0.195	0.010	5.3	0.016	12.2	27.5
	3	11:40–12:40	0.117	0.005	4.6	0.020	5.9	29.2
	4	12:40– 1:37	0.058	0.002	4.2	0.024	2.4	29.2
C Aerated	1	9:40–10:43	0.354	0.026	7.1	0.008	44.3	26.0
	2	10:43–11:41	0.346	0.017	4.9	0.010	34.6	27.5
	3	11:41–12:40	0.265	0.011	4.3	0.014	18.9	29.2
	4	12:40– 1:39	0.137	0.007	5.0	0.017	7.9	29.2
D Aerated	1	9:42–10:44	0.197	0.011	5.5	0.006	32.8	26.0
	2	10:44–11:47	0.141	0.006	4.3	0.010	14.1	27.5
	3	11:47–12:45	0.118	0.004	3.8	0.013	9.1	29.2
	4	12:45– 1:42	0.128	0.005	4.2	0.015	8.5	29.2
C & *D* Aerated	1	2:00– 3:00	0.599	0.025	4.1	0.063	9.5	32.5
	2	3:00– 4:00	0.483	0.017	3.6	0.064	7.6	31.0
	3	4:00– 5:00	0.542	0.025	4.6	0.066	8.2	29.0
	4	5:00– 6:00	0.554	0.014	2.5	0.066	8.4	27.0
	5	6:00– 7:00	0.531	0.011	2.0	0.066	8.0	25.0
	6	7:00– 8:00	0.494	0.010	2.1	0.063	7.8	23.0
	7	8:00– 9:00	0.776	0.025	3.3	0.061	12.7	21.0
	8	9:00–10:00	1.155	0.041	3.6	0.064	18.1	20.0

* The values in this column are calculated rates based on the cross-sectional area of the total phloem. Since the sieve tubes occupy 25 per cent. or less of the phloem, these values must be multiplied by a factor of at least 4 to give actual rates.

maintained for many hours; and although the rate may fluctuate considerably, there is no indication of a complete stopping. During the 12 hour period, incidentally, there was collected from the plants in aerated culture solution an average volume of phloem sap equivalent to the *total volume of phloem* in 173 cm. of stem. About 36 cm. of stem were removed in the form of thin slices. The temperature and humidity in the greenhouse were such that even the plants in aerated culture solution were wilted slightly between 2:30 and 5:00 P. M.

In order further to confirm these results, a second test was made using two new plants in aerated culture solution. Two vigorous young plants, as

nearly alike as possible, were selected. Collections were started at 2:00 P. M. on July 21, 1934. One plant had sixteen nodes, the other seventeen, and each was about 8 feet in length.

At 7:00 P. M. the steam heat was turned on in the greenhouse, and at 7:30 four 500-candlepower floodlights were hung above the plants. Because of the steam and also the radiation from the lights, the greenhouse temperature remained relatively high; about midnight the leaves on one plant were slightly wilted. Two of the lights, consequently, were turned off; and at 2:00 A. M. a ventilator was opened. As the temperature was still 25° C. at 4:00 A. M., two more ventilators were opened; by 4:30 it had dropped to 20° C. Soon the sap started to come faster from both plants, and by 5:00 there was active xylem exudation. This continued for 2 hours and then stopped as the light intensity increased. Phloem exudation also dropped until 10:00 A. M., then it started to increase, and continued to gain in volume until the end of the run. The data from this test appear in table II.

The dry weight composition of the sap collected between 4:00 and 8:00 A. M. indicates that the sap was diluted with xylem exudate. To give a more correct picture of the rates of flow during this period, the values in the rate column have been corrected on the basis of a dry weight composition of 3.7, which is the average for the periods 3:00–4:00 A. M. and 8:00–9:00 A. M. Even with this correction the rates are high, as might be expected on the basis of the relative abundance of water. Under these conditions an osmotic system would tend to absorb water faster, flow would be increased, and solutes would be lost faster than they were being supplied. Next would follow a period of slow exudation as the water became less available. That this is the case from 8:00 until 12:00 is shown lower in this column (table II). These stems were about 240 cm. in length at the start of the experiment. Approximately 72 cm. were removed during the 24-hour collection period. A volume of sap equivalent to the *total phloem volume* in 189.9 cm. of stem was collected. This figure is based on the corrected values given in the rate column.

A third set of collections that was made seems to render even more improbable the idea that phloem exudation is caused by cell turgor or by the release of tissue tensions. In these tests the stems were first steamed below the cotyledons and cut from the roots, which again were in aerated culture solutions. Three groups of four plants each were used. In one group the excised stem of each plant was cut near the tip so that the apical portion represented about 25 per cent. of the stem and the basal portion 75 per cent. In the second group the apical portion was the larger and the basal portion relatively small. In the third group an attempt was made to cut the stem with two portions of equal weight. The stems were handled individually, and after they were cut, sap was collected from the apical and basal portions

simultaneously as long as it flowed freely. The cut was made in the center of an internode in each case. Data on these collections appear in table III.

TABLE II

PHLOEM EXUDATION FROM SQUASH. VOLUME AND RATE OF FLOW FROM TWO WATER CULTURE PLANTS FOR 24 HOUR PERIOD. PLANTS WITH SIXTEEN AND SEVENTEEN DEVELOPED NODES, APPROXIMATELY 8 FEET IN LENGTH. COLLECTIONS MADE JULY 21, 1934

COLLECTION		WEIGHT OF SAP		DRY WT. AS PERCENTAGE OF FRESH	PHLOEM AREA	LINEAR* RATE OF FLOW PER HR.	TEMPERATURE OF GREENHOUSE
No.	TIME	FRESH	DRY				
		gm.	*gm.*	%	*cm.*^{2}	*cm.*	°*C.*
1	2:00– 3:00	0.788	0.055	6.9	0.044	17.9	26.0
2	3:00– 4:00	0.852	0.042	4.9	0.059	14.4	27.0
3	4:00– 5:00	0.710	0.032	4.5	0.060	11.8	22.5
4	5:00– 6:00	0.596	0.026	4.4	0.060	9.9	30.0
5	6:00– 7:00	0.437	0.019	4.3	0.073	6.0	26.0
6	7:00– 8:00	0.321	0.016	5.0	0.077	4.2	21.0
7	8:00– 9:00	0.499	0.020	4.1	0.077	6.5	24.0
8	9:00–10:00	0.671	0.024	3.6	0.077	8.7	23.5
9	10:00–11:00	0.725	0.023	3.1	0.084	8.6	24.5
10	11:00–12:00	0.584	0.019	3.3	0.087	6.7	23.0
11	12:00– 1:00	0.586	0.018	3.2	0.082	7.1	23.5
12	1:00– 2:00	0.689	0.021	3.0	0.082	8.4	25.0
13	2:00– 3:00	0.775	0.022	2.9	0.082	9.4	22.0
14	3:00– 4:00	0.644	0.021	3.3	0.086	7.5	25.0
15	4:00– 5:00	1.722	0.031	1.8	0.087	9.7	20.5
16	5:00– 6:00	2.622	0.037	1.4	0.087	11.5	23.5
17	6:00– 7:00	2.560	0.034	1.3	0.087	10.6	23.0
18	7:00– 8:00	0.507	0.014	2.8	0.087	4.4	23.0
19	8:00– 9:00	0.185	0.008	4.2	0.087	2.1	23.3
20	9:00–10:00	0.179	0.007	4.1	0.087	2.1	24.5
21	10:00–11:00	0.265	0.013	4.9	0.091	2.9	26.1
22	11:00–12:00	0.251	0.011	4.5	0.088	2.9	28.7
23	12:00– 1:00	0.648	0.023	3.6	0.086	7.5	29.5
24	1:00– 2:00	0.766	0.028	3.7	0.086	8.9	30.0

* The values in this column are calculated rates based on the cross-sectional area of the total phloem. Since the sieve tubes occupy 25 per cent. or less of the phloem, these values must be multiplied by a factor of at least 4 to give actual rates.

As these figures (table III) show, the volume of sap exuding from a portion of stem was roughly proportional to the weight of the stem; and it was not the nature of the local tissues but the mass of the stem acting as a reservoir and source of sap that regulated the volume delivered. The two halves of the cut internode were practically identical in every case, so that the first

TABLE III

RELATIVE VOLUMES FROM TIP AND BASAL PORTIONS OF EXCISED STEMS. COLLECTIONS MADE JULY 27, 1934. TEMPERATURE 22°–29° C. IN HEAD HOUSE

STEM NO.	TIME	LENGTH OF STEM		NO. OF LEAVES		FRESH WEIGHT		FESH WEIGHT OF SAP		DRY WEIGHT OF SAP		DRY WEIGHT AS PERCENTAGE OF FRESH	
		TIP	BASE	TIP	BASE	TIP	BASE	TIP	BASE	TIP	BASE	TIP	BASE
	min.	*cm.*	*cm.*			*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	%	%
1	68	60	160	4	13	52	479	0.191	0.452	0.016	0.037	8.4	8.3
2	45	40	200	2	12	20	322	0.048	0.186	0.005	0.016	9.6	8.5
3	30	40	135	3	9	26	327	0.117	0.438	0.010	0.035	8.6	7.9
4	30	45	170	3	9	20	305	0.061	0.193	0.006	0.017	9.7	8.7
5	22	190	10	24	1	630	68	0.239	0.075	0.018	0.005	7.7	6.9
6	30	210	50	11	3	236	114	0.322	0.164	0.025	0.012	7.7	7.1
7	50	180	40	10	2	204	62	0.274	0.136	0.024	0.010	8.7	7.3
8	30	200	25	12	2	288	75	0.260	0.071	0.023	0.006	8.7	8.5
9	30	140	20	14	2	153	100	0.189	0.177	0.014	0.012	7.5	6.6
10	30	90	40	9	4	112	93	0.137	0.101	0.012	0.008	8.5	7.9
11	30	110	75	6	7	190	190	0.218	0.222	0.020	0.018	9.1	8.5
12	30	80	70	8	5	95	121	0.245	0.269	0.026	0.023	10.6	8.7

5 cm. or more of stem from the cut was the same. It was the tissue beyond the first node, therefore, that determined the amount of sap collected.

In this series two additional points of interest were noted. Ordinarily the most rapid flow occurs from the young growing tissues near the apex of the stem (**4**). When the apical portions of these stems had been allowed to exude from their basal ends until flow had practically ceased, a cut near the tip did not result in exudation from that region. The stems acted as though they had been exhausted, and no more sap could be obtained by cutting.

The second point is apparent in the last two columns of table III. In every case the dry weight concentration of the exudate from the tip portion exceeded that from the base. This fact is further evidence for a concentration gradient in the actual conducting channels (**4**, table XIII, p. 210). There is no question of an admixture of saps in these experiments, since the shoots used were all excised and wilted soon after their removal from the root systems.

One further set of collections that was made gives some idea of the nature of xylem exudation. This experiment was run in conjunction with T. C. BROYER, Division of Plant Nutrition, who made most of the collections. The object of the experiment was to find the volume of xylem exudate that could be collected in relation to the size of the root system.

The plants were young squash grown in aerated, full nutrient culture solution. The four used in this test were removed from the culture solution, the roots washed, and the plants set up in tap water at 9:00 A. M., July 25. They were cut just below the cotyledons at 9:15, and the collections started. These continued until 9:00 A. M., July 26. The roots were then removed, centrifuged free of excess water, and weighed. Their total weight was 450 gm. During the 24 hours, 551.7 cc. of exudate were collected. These roots therefore exuded in this period a volume of sap greater than their own volume. Since less than 20 per cent. of this total volume is occupied by the lumina of the xylem vessels, evidently these conducting elements had their contents displaced more than five times during the test. The results of this experiment are shown in table IV.

It seems evident that large samples of xylem sap can be obtained from excised root systems of squash.

After these quantitative tests, a series of qualitative observations and microscopic studies were made in an attempt to explain the nature of stoppage of the phloem, together with the relation of sieve tube inclusions to flow and stoppage, and to compare in several ways phloem and xylem exudates.

In the comparisons the following points were observed: The dry weight composition of xylem exudate from a group of plants in aerated culture

TABLE IV

VOLUME AND COMPOSITION OF XYLEM EXUDATE FROM SQUASH COLLECTED JULY 7, 1934, AT BERKELEY, CALIFORNIA

No.	SAP COLLECTION		RATE PER PLANT PER MIN.	PERCENTAGE DRY MATTER	TEMPERATURE	
	TIME	VOLUME			CULTURE SOLUTION	GREENHOUSE
		cc.	*cc.*	%	°C.	°C.
1	9:15– 9:30	10.5	0.17	0.16	25.0	26.5
2	9:30– 9:45	28.0	0.47	0.16	.....	.....
3	9:45–10:00	30.0	0.50	0.16	.....	.....
4	10:00–10:10	24.5	0.61	0.15	.....	.....
5	10:10–10:15	10.2	0.51	0.13	.....	.....
6	10:15–10:20	12.5	0.62	0.16	.....	.....
7	10:20–10:25	12.0	0.60	0.18	.....	.....
8	10:25–10:30	12.5	0.62	0.18	.....	.....
9	10:30–10:35	12.5	0.62	0.19	.....	.....
10	10:35–10:40	11.5	0.57	0.22	.....	.....
11	10:40–10:45	10.5	0.52	0.18	25.0	30.0
12	10:45–10:50	11.0	0.55	0.19	.....	.....
13	10:50–10:55	10.0	0.50	0.20	.....	.....
14	10:55–11:00	8.5	0.42	0.23	.....	.....
15	11:00–11:05	9.5	0.47	0.21	.....	.....
16	11:05–11:10	9.5	0.47	0.23	.....	.....
17	11:10–11:20	16.0	0.40	0.21	.....	.....
18	11:20–11:30	14.5	0.36	0.19	26.0	30.5
19	11:30–11:40	12.5	0.31	0.21	.....	.....
20	11:40–11:50	10.5	0.26	0.22	.....	.....
21	11:50–12:00	9.0	0.22	0.21	.....	.....
22	12:00–12:15	11.5	0.19	0.20	.....	.....
23	12:15–12:30	9.5	0.16	0.18	28.3	32.0
24	12:30–12:45	8.0	0.13	0.16	.....	.....
25	12:45– 1:00	7.5	0.12	0.22	.....	.....
26	1:00– 1:30	10.5	0.09	0.18	.....	.....
27	1:30– 2:00	10.0	0.08	0.14	30.0	33.0
28	2:00– 2:30	9.0	0.08	0.15	.....	.....
29	2:30– 3:00	8.0	0.07	0.14	31.3	33.3
30	3:00– 3:30	8.5	0.07	0.13	.....	.....
31	3:30– 4:00	8.0	0.07	0.15	31.5	33.0
32	4:00– 4:30	7.0	0.06	0.15	.....	.....
33	4:30– 5:00	7.5	0.06	0.13	.....	.....
34	5:00–10:00	70.0	0.06	0.12	31.5	32.0
35	10:00– 8:00	20.5*	0.03	0.11	24.2	16.5
36	" "	13.0*	0.02	0.17	.....	.....
37	" "	24.0*	0.04	0.14	.....	.....
38	" "	29.0*	0.05	0.14	.....	.....
39	8:00– 9:00	6.5	0.03	0.10	18.0	23.0

* Volumes collected from the four separate plants from 10:00 P. M. on July 7 until 8:00 A. M. on July 8.

solution was 0.59 per cent.; from plants in non-aerated culture solution, 0.49 per cent.; and from a relatively pure tap water, 0.21 per cent.

Phloem exudate under these three conditions showed no consistent differences. On the other hand, from these young, vigorously growing squash plants in the blossoming stage the phloem sap averaged about 4 per cent. dry weight, whereas that from fruiting cucumber and pumpkin plants ran more than 10 per cent. in many cases during the first few minutes (**2, 4**).

Phloem sap has been found always to coagulate. This process takes place very rapidly upon heating or treating with alcohol; slowly in the cold in stoppered test tubes. The coagulum will not dissolve in mild acid or alkali. In no case has the xylem sap been found to coagulate.

Exudate collected from the phloem of cucurbits used in these experiments flowed out at rates of from 0.01 to 0.1 cc. per minute. While xylem sap from the same and similar stems commonly flows at many times this rate and will continue for hours with little change, the phloem soon plugs and must be reopened by cutting.

Phloem sap may be coagulated in the stem by heat; when the heated portion is cut, no phloem exudation occurs. Heating had no visible effect upon xylem exudation.

Exudation from the cut xylem depends upon conditions affecting water loss. Even when only the excised root system is involved, water deficit must be satisfied before an actual exudation occurs. Guttation occurs only when conditions tend to reduce transpiration to a value lower than that of water absorption by the roots.

Phloem exudation will occur from severely wilted plants. The volume is reduced in proportion to the water deficit, but the dry weight composition tends to increase in compensation.

Phloem sap from the plants studied at Berkeley had a pH of approximately 7.3, as shown by spot-plate tests with indicators. It is rather highly buffered. Xylem sap from the same plants had a pH of 5.5 and was practically unbuffered.

Additional information was gained from microscopic tests on phloem tissues. Slime plugs result from coagulation of the disintegration products of the nucleus and slime-drops in cucurbits (**4**). Consequently, they can be found only in sieve tubes during a certain stage in their ontogeny. This stage corresponds, apparently, with that at which loss of the property of semipermeability takes place. Possibly, therefore, slime plugs may occur naturally in sieve tubes in which sap flow is very rapid. They always occur in these tubes in cut stems if exudation has been rapid and may be found at a distance of 10 cm. or more from the cut surface, lodged against the distal sides of sieve plates. An interesting fact is that although these structures are present for many centimeters from the cut, removal of a slice as thin as

0.5 mm. will cause exudation to resume after stoppage. Apparently they occur only in tubes in a certain stage of maturity and have little or nothing to do with stoppage.

When a stem of squash is freshly cut and is wiped repeatedly, the large central tubes of the phloem groups are clogged first, and flow continues longest from the peripheral and cortical tubes (**4**). If stoppage were caused by plugging of the sieve tube lumina, just the opposite would be expected. Repeated microscopic examinations have failed, furthermore, to show the lumina of cut sieve tubes, from phloem in which flow has ceased, to be filled with coagulated sap. In many cases the only evidence of stoppage was a peculiar staining of the walls of the *cut sieve tube elements.* Upon second thought, this seems to answer logically the question regarding the nature of phloem stoppage. From the standpoint of hydraulics it seems highly improbable that flow from a cut tube under a head of several atmospheres could be stopped within the lumen of the tube by a slow coagulation of the flowing sap. It would be washed away faster than it could possibly accumulate. Furthermore, stoppage of flow in intact stems with heat does not cause a plugging of the lumina, but on the contrary, usually fixes the contents in place, and in sieve tubes past the slime plug stage may leave them relatively free of coagulum.

The coagulated phloem sap has an affinity for aniline blue and gives a peculiar violet color reaction. In the walls of the actual cut elements this same color reaction occurs.

It seems, therefore, that stoppage results from coagulation of the phloem sap within the interstices of the walls of the cut elements. Again the possibility occurs that normal flow across end walls of the sieve tubes takes place not through tubules or perforations in the protoplasmic connections but through the intermolecular spaces of the cellulose walls.

Discussion

The experiments described indicate that phloem exudation, rather than being of local origin and caused by turgor or release of tissue tensions, is a direct manifestation of a process occurring throughout the plant. As a result of this process, organic foods are moved over long distances in relatively short times. The reactions of this conducting system to manipulation indicate it to be of the nature of an elongated, somewhat elastic osmometer working under a positive pressure and activated by a concentration gradient of its osmotically active contents.

The mechanism of xylem exudation, on the other hand, seems to be of a different nature. While the commonly pictured osmotic mechanism might explain a slow absorption of water, it cannot account for the rapid flow obtained from the plants used in the experiment reported in table IV. The

xylem vessels of these root systems had their total contents replaced many times within the period of 24 hours. Furthermore any theory of root pressure which fails to explain the simultaneous absorption and secretion into the non-living xylem vessels of water and inorganic ions is inadequate, for although these two functions may not be directly correlated, in the living plant they are both in process at the same time and they involve the same tissues.

While the xylem and phloem constitute two separate and distinct systems within the plant, either or both of which may exhibit exudation, the fact that the former may act as a source of water for the osmotic phloem system imposes upon them a certain interdependence. Situated as they are in close proximity over a relatively large area and separated by only a thin layer of cells, a very delicate water balance must necessarily exist between them. The open nature of the xylem should effect a rapid adjustment to fluctuating pressure conditions throughout the system. Such changes in pressure, therefore, would be transmitted to and effective upon the total phloem system at approximately the same time. Movement within the phloem, on the other hand, is relatively slow, with much internal resistance, and in addition the phloem gives every evidence of acting like an inflated elastic system. Consequently response to fluctuations in pressure in the phloem would be slower and changes in rate would be gradual.

With this relationship in mind, it might be interesting to consider briefly what happens when the cucurbit stem is cut and the phloem exudation occurs. Granting that translocation is occurring normally under a gradient of pressure within the phloem, and that transpiration is maintaining a subatmospheric pressure within the xylem, when the stem is cut a rapid readjustment must occur. First, cutting opens the phloem, allowing loss of sap against only atmospheric pressure. The gradient in pressure is greatly steepened and internal pressure rapidly lowered by exudation. In the xylem, resistance to uptake of water is removed and xylem sap flows rapidly away from the cut end until the whole xylem system comes approximately to atmospheric pressure. Water is relatively abundant for uptake by the phloem. Not only the xylem but all living cells in contact with the phloem serve as a source of readily available water because of the decrease in turgor pressure and consequent increase in suction force in the phloem.

The result of these combined changes is to produce an abnormally high rate of flow from the phloem which may be ten or more times the normal rate (**4**). With the deflation of the elastic system and the readjustment of water relations, this high rate falls off rapidly and at the same time concentration of the exudation sap decreases. Previous data (**4**, table X) indicate that 15 minutes or more may be required for the establishment of new equilibrium conditions; and the initial high rate and concentration of the exudate result in high average values during the first hour of collection, as shown in tables I and II.

With clogging of the xylem vessels with air, subatmospheric pressure is again established, and with lowered water availability pressure in the phloem is further lowered and several hours may be required for exudation flow to become normal again. If the supply of organic nutrients to the phloem is maintained there should be an increase in concentration to compensate for the lowered rate of flow.

In interpreting data on extended collections, as given in table II, even other factors must be considered. The lowering concentration of the exudate from sundown until daylight the following morning is probably related to the supply of soluble materials to the phloem. The great increase in rate shown in both tables I and II is related, on the other hand, to decreased transpiration. In both experiments water absorption by the roots exceeded water loss for a period and active xylem exudation resulted.

Following this, as transpiration again lowered water availability, the rate was greatly decreased and the concentration gradually built up. With increasing supply of osmotically active solutes, rate and concentration both approached again the values attained the previous day after the initial period. It should be pointed out that the initial rate of exudation from cut phloem is strictly abnormal owing to rapid deflation of an expanded system. The concentration at this time is very near normal. On the other hand, concentration after cutting cannot again attain the normal value so long as the system is kept open because of the new pressure conditions. Soluble materials are carried to the open end of the system as fast as they are supplied, and the original turgid condition with its attendant high concentration is not attained.

The interdependence of concentration and rate that is so intimately related to water availability is again brought out in a consideration of the effects of diurnal pressure variations in the xylem. With the lowering of xylem pressure by transpiration, flow of water to the phloem decreases, concentration of the phloem sap increases (if the supply of solutes is maintained), and translocation continues at somewhat lowered linear rates of flow. With increased xylem pressures at night, an increase in exudation pressure occurs and translocation takes place at a higher rate and lowered concentration. Because of this ready exchange of water across the intervening tissues, the osmotic phloem system responsible for phloem exudation can maintain translocation regardless of pressure conditions in the xylem, provided a supply of osmotically active materials is maintained.

Xylem exudation is of common occurrence in the spring. JAMES and BAKER (9) question its presence in any plants except the grape. I have found it easy to demonstrate in the common annuals that grow rapidly in March and April. *Brassica arvensis* (L.) B.S.P., *Malva parviflora* L., and *Lupinus bicolor* Lindl. make convenient demonstration material. Undoubt-

edly hundreds of other species, including many trees and vines, show this same phenomenon at certain times. That the flow actually occurs from the xylem can readily be shown by peeling back the cortical tissues and scraping off the cambium. In the vine it has been demonstrated even in some of the inner annual rings, while in the maple it occurs principally in the sapwood.

One might consider that in the trees and vines showing xylem exudation the situation is different from that in the leafy annuals mentioned. Examination of vines showed, however, that the roots of these plants were active and in some cases were actually producing many new feeding laterals, even though the buds were scarcely swelling. Apparently, therefore, the cases are similar; that is, an active root system is absorbing water faster than it is being lost, thus giving rise to a visible exudation of sap. In the leafy annuals the tops have to be removed before this exudation can be shown. In the woody species, complete removal of the top growth will eliminate the transpiration loss, and root pressure may be demonstrated well into the summer season. The fact that rapid hydrolysis of stored carbohydrates may result in a certain loss of sugars into this stream of xylem sap apparently has no direct bearing on the type of mechanism involved. The source of pressure seems to be in the active root tips, which are only remotely connected with this solution. The rate at which water may be absorbed and the location of the active organs seem to preclude the possibility that this sugar solution acts osmotically to cause water absorption from the soil.

There seems to be little justification for the attempt by JAMES and BAKER (9, p. 341) to assume an "essential unity of all sap movements in the plant" and to "include" these separate phenomena under "the common name of sap pressure." Such an assumption can only lead to error. Likewise the suggestion of STEWARD and PRIESTLEY that phloem exudation is strictly local in origin seems unsound in the light of the evidence set forth in this paper.

The recently proposed mechanism of VAN DEN HONERT (18) to explain translocation of foods on the basis of a surface tension phenomenon, although interesting for purposes of speculation, seems to have little support from the internal structure of phloem tissues. VAN DEN HONERT'S suggestion of the protoplasm vacuole interface as constituting the channel will scarcely bear scrutiny. When the sieve tubes are young and this interface is prominent, the cell has semipermeable properties, and movement from cell to cell would be difficult to explain. As the sieve tube elements mature, this interface appears to break down (5) and to give way to a region of indefinite properties. The protoplasmic strands of the sieve plate remain intact however, and the vacuoles consequently continue discrete in the separate elements, so that movement from cell to cell could not follow such an interface. The protoplasm ceases its streaming movements and apparently enters a phase of lowered activity.

Finally, from the standpoint of volume relations, such a mechanism seems highly improbable. Although it might explain movement of hormones in minute quantities, provided a continuous interface could be demonstrated, it would fail, as would protoplasmic streaming (5), to provide adequate transport for the quantities of food materials normally moved in plants.

I wish to express my appreciation to Professor D. R. HOAGLAND for the use of plants and greenhouse facilities required for these experiments, and to Mr. T. C. BROYER for help with the collections and for data included in table IV.

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UTILIZATION OF GALACTOSE BY *ASPERGILLUS NIGER* AND *PENICILLIUM GLAUCUM*

W. H. Horr

(WITH FIFTEEN FIGURES)

Literature

In working with the hexose sugar galactose, Knudson (**12**) found that in concentrations of 1–2 per cent. it was toxic to the seedlings of *Vicia villosa* and *Pisum sativum*. This toxicity was evidenced by a browning and killing of the roots. He also found that this toxicity was prevented by the addition of an equal concentration of dextrose to the solution. Knudson suggested that this might be brought about by a polymerization of the two sugars but he found no trace of lactose in the remaining solution. He also suggested that the toxicity might be due to oxidation products of galactose, and that glucose might prevent their absorption or modify their action. In a later paper (**13**) he found that galactose was toxic in concentrations as low as 0.0125 molecular solution, and that this toxicity was neutralized by saccharose, glucose, levulose, and lactose in 0.025 molecular concentration. He also found that mannose was toxic in 0.025 molecular solution and that this was neutralized by dextrose in an equal concentration. He states that mannose is not so toxic as galactose, and suggests that the dextrose might prevent the absorption of the galactose. Working with other sugars he found that sucrose and lactose caused a decided acceleration in the growth of timothy, and expressed the opinion that lactose is probably not assimilated by timothy; but he offered no explanation of this acceleration produced by the presence of lactose.

Similar effects of galactose on green seedlings were reported by Heinonen (**10**), who repeated the work of Knudson and carried the studies further. She, too, found that dextrose neutralizes the toxicity of galactose, but that this neutralizing effect held only until the seedling used all of the dextrose in the solution and then there was a toxic action quite like that when pure galactose was supplied. It took several months, however, for the dextrose to be completely consumed and the toxicity to appear. She described the manifestations of this toxicity as bulbous enlargements of the root tips accompanied by a progressive brown discoloration of the epidermal and subepidermal tissues. She believed Knudson's suggestion that the dextrose prevented the absorption of galactose to be right, but did not present any sugar determinations on the used culture solutions to support this belief. She concluded that these were general non-specific responses, to be expected with toxins of various kinds, because of similar responses obtained by Sommer and Sorokin (**28, 29**) in their work on the deficiency of essential

elements. NIGHTINGALE *et al.* (**21**) reported a similar bulbous enlargement of root tips and a browning of the tissues followed by a general tissue sloughing in seedlings grown in a medium deficient in calcium. They did not state that these effects were due to a toxic action.

That other sugars may be toxic to green plants and their toxicity neutralized by the addition of a different sugar, is shown by the work of BRANNON (**2**), who found that fructose alone is toxic to peas and that its toxicity is neutralized by the presence of glucose. As he made no statement as to the nature of this toxicity, the effects cannot be compared with those noted by KNUDSON and HEINONEN.

The neutralizing effect of sugars on a non-sugar toxin is evidenced by the report of BOAS and MERKENSCHLAGER (**1**), who found that galactose is best and xylose second best, of the sugars which they used, for counteracting lime toxicity to *Lupinus luteus*.

One also finds that galactose may be utilizable to some degree, or be entirely unusable by some green plants other than those used by KNUDSON and HEINONEN. BURGE, WICKWIRE, ESTES, and WILLIAMS (**3**) found that *Spirogyra* and *Paramaecium* were able to utilize galactose, but not so well as they did dextrose and levulose. Similar results were obtained by ROBBINS (**24**) in his work on *Ceratodon purpureus*. This plant absorbed and used sucrose, levulose, maltose, dextrose, lactose, and galactose for growth and starch formation, but only a slight development was obtained when galactose and lactose were used as a source of carbon. These plants were unable to use mannite, glycerol, or starch. The experiments were carried on in darkness and no normal development of plants was obtained. ROACH (**23**) obtained a rapid growth of *Scenedesmus* on galactose, saccharose, glucose, and maltose, a moderate growth on levulose, but only a slight growth on mannite. With *Cystococcus* she obtained a rapid growth on mannite, glucose, fructose, and saccharose, but only a moderate growth on galactose and levulose.

According to the results of TERROINE and coworkers (**30**), peanut embryos were unable to utilize galactose, lactose, xylose, or arabinose, but they made a good uniform growth when supplied with dextrose, levulose, sucrose, or maltose. In this work there is no mention of any toxic effect from any of the non-assimilable sugars.

The carbohydrate-using non-green plants seem to be more tolerant toward different sugars. Their ability to utilize galactose as a source of carbon varies with the plant, but no cases of toxicity were found. PETERSON and FRED (**22**) report that *Lactobacillus pentoaceticus* was able to utilize galactose and dextrose with equal ease. Fifteen isolations of *Rhizoctonia* studied by MATSUMOTO (**17**) grew equally well on galactose and fructose, and in addition were able to use starch and amygdalin as carbon sources. *Plenodomus fuscomaculus* when grown by COONS (**4**) on M/20 dextrose and M/20 galac-

tose developed the same sized colonies, 2 mm. in diameter, and had the same form of fructification, oidia, at the end of two months. On raffinose, loose floccose colonies were developed which were larger than those produced on M/20 xylose. These were of solid compact growth, and the form of fructification was oidia. Lactose produced no growth at all.

KENDALL (**11**), working on the identification of carbohydrates by bacterial procedure, found only two of the bacteria he used (*Bacillus mesentericus* and *Vibrio* of Finkler and Prior) which were unable to ferment galactose.

With these varying data in mind the work described in this paper was undertaken. An attempt was made to determine: (1) whether or not galactose is toxic to some fungi as well as to some green plants; (2) if so what is the nature of this toxicity? (3) if there is a neutralization of toxicity by the presence of other sugars, how is this produced?

Methods

The stock cultures of the organisms used in these experiments, *Aspergillus niger* van Tiegh and *Penicillium glaucum* Link, were started from single spore cultures. The mineral nutrient medium used was COON'S "cheap synthetic solution" as described by YOUNG and BENNETT (**32**), to which 2 per cent. of sugar was added, except in some special tests. The solutions were sterilized in an Arnold steam sterilizer.

This concentration of sugar was used because it provided enough carbohydrate to prevent autolysis during the growth periods used, and according to NIETHAMMER'S (**20**) work with *Aspergillus niger* on sucrose it is well within the range of sugar tolerance of that organism.

The cultures were grown at 20° C. as that temperature was within the growth range of both organisms and there was a room available in which that temperature was maintained.

The rate of growth was taken from the dry weight of the mycelium formed. This was measured by filtering and drying the mycelium mats in Gooch crucibles. This method does not give an accurate measure because of the loss of spores through the filter. Porcelain filters which retained the spores were tried but they soon became clogged, and the rate of filtration was so reduced that they were impractical. Two or three flasks were used in each case and all the tests repeated several times.

Some linear growth rate measurements were made on agar in petri dishes, and on microscopic cover glasses. All pH readings were made with a Leeds and Northrup quinhydrone pH indicator. Before making the potentiometric determinations, the filtrate was boiled to remove the excess carbon dioxide.

The sugars used were prepared by the Pfanstiehl Chemical Company

and examined and declared pure by the United States Bureau of Standards, Department of Chemistry.

In setting up a series of culture flasks, a sufficient volume of mineral nutrient was made up to fill all flasks. From this enough dextrose and galactose solutions were made to make all pure solutions as well as the mixed solutions, thus insuring uniformity of the sugars.

All inoculations for a series were made at one time, using 1 cc. of a heavy spore suspension to insure a heavy uniform growth on each of the flasks, and each set within a series was filtered after the designated time had elapsed.

Experimentation

The strain of *Aspergillus niger* used in these experiments, while able to utilize galactose and mannose and develop both mycelium and spores, does not utilize either as readily as it does dextrose and levulose. With mixtures of galactose and dextrose, there was noted an acceleration of growth which caused, in some mixtures, a considerable increase in the dry weight of the mycelium produced. This acceleration in growth has always indicated two optimum concentrations of the individual sugars in the mixed solutions. The mixtures giving the optimum growth seem to vary with other conditions (fig. 1).

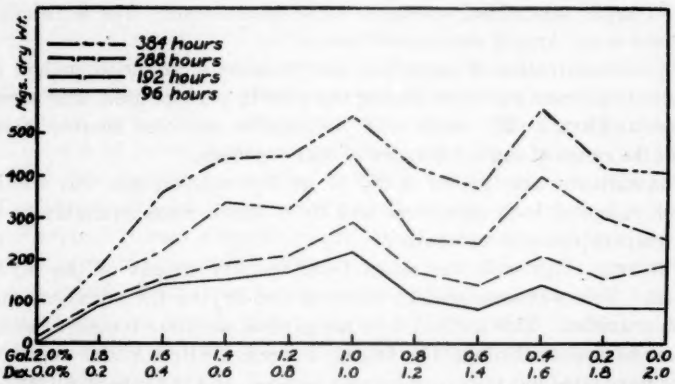

FIG. 1. *A. niger* grown at room temperature; 100 cc. of solution in each of two 125-cc. flasks.

In figure 2, where the organisms were given a larger surface area per unit of culture medium upon which to grow, the greatest acceleration occurred in the more dilute concentrations of galactose.

A similar growth reaction to mixtures of these sugars is exhibited by *Penicillium glaucum*, but as this organism utilizes the galactose much more readily than does *Aspergillus niger*, the acceleration is not so marked. It is

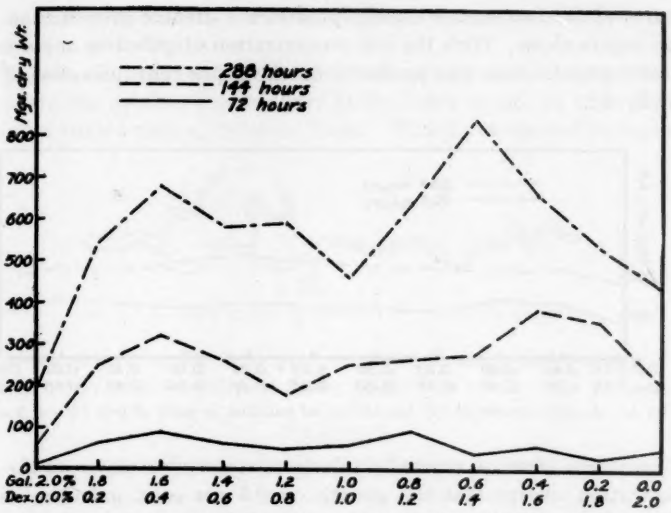

FIG. 2. *A. niger* grown at 20° C.; 100 cc. of solution in each of two 250-cc. flasks.

interesting to note that in nearly every case there is a greater amount of dry weight produced on the mixed sugars than is produced on the 2 per cent. dextrose solution (fig. 3).

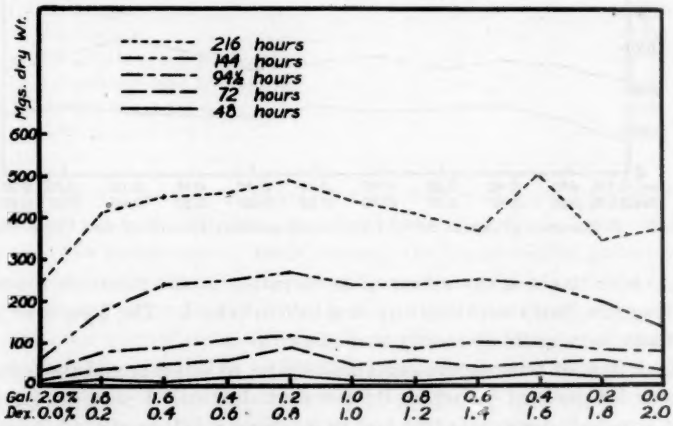

FIG. 3. *P. glaucum* grown at 20° C.; 100 cc. of solution in each of two 125-cc. flasks.

The total sugar concentration in each solution was reduced to 0.5 per cent. to determine the growth rates on low sugar concentrations. Again the

mixtures of the two sugars usually produced a greater growth than either of the sugars alone. With the low concentration of galactose there is often a greater growth than was produced on the 2 per cent. galactose of other tests (fig. 4).

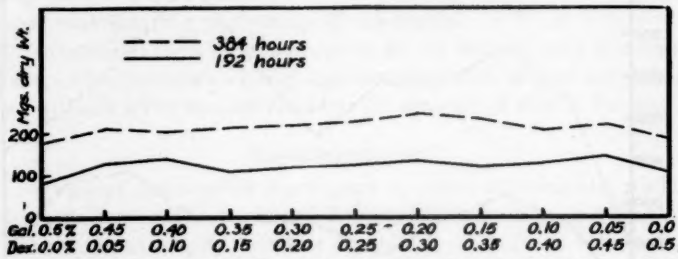

FIG. 4. *A. niger* grown at 20° C.; 100 cc. of solution in each of two 125-cc. flasks.

Penicillium glaucum reacted similarly to *Aspergillus niger* on the dilute concentration, except that the growth on 0.5 per cent. galactose solution gave a smaller growth than was produced on the 2 per cent. galactose solution (fig. 5).

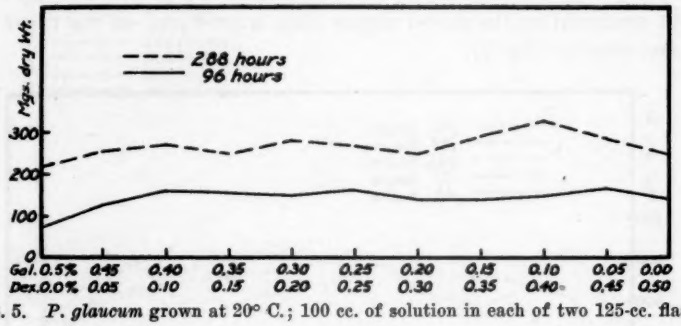

FIG. 5. *P. glaucum* grown at 20° C.; 100 cc. of solution in each of two 125-cc. flasks.

In order to check the extent of acceleration in the solutions containing mixed sugars, flasks were made up as shown in table I. The 2 per cent. galactose flasks were made up merely as a check.

Each flask of pure sugars contained 100 cc. of solution and the tests were made on 1.8 per cent. galactose, 0.2 per cent. dextrose, 1 per cent. galactose, and 1 per cent. dextrose, alone and in mixtures. Where the solutions were mixed, four flasks, each containing 100 cc. of solution, were used to provide a growing surface and a total amount of sugar equal to that provided when each of the sugars was in a separate flask and two flasks used for each sugar.

Thus the surface exposed for growth and the total amounts of sugar available were equal in each comparison.[1]

When the solution contained 1.8 per cent. galactose and 0.2 per cent. dextrose, there was produced three times as much dry weight as when the same amounts of sugars were in different flasks. With 1 per cent. of each sugar in

FIG. 6. *A. niger* grown 65 hours at 28° C. on 2% galactose.

the solution there was two and one-half times as much dry weight as when the sugars were used in different flasks (table I).

TABLE I

ASPERGILLUS NIGER, GROWN 168 HOURS AT 20° C.

SUGAR	NUMBER OF FLASKS	DRY WEIGHT
%		*gm.*
1. 2 galactose	2	0.0443
2. 1.8 galactose	2	0.0424
3. 1.0 galactose	2	0.0451
4. 1.0 dextrose	2	0.4110
5. 0.2 dextrose	2	0.1456
6. 1.8 galactose and 0.2 dextrose	4	0.5774
7. 1.0 galactose and 1.0 dextrose	4	1.1516
Adding nos. 3 and 4	....	0.4561
Adding nos. 2 and 5	....	0.1880

Linear growth was investigated on 2 per cent. galactose, 2 per cent. dextrose, and 1 per cent. galactose plus 1 per cent. dextrose in agar petri dish cultures. The measurements made were on the heavy visible portion of the mat without considering the microscopic mycelium which extended beyond. With 2 per cent. dextrose, and with 1 per cent. galactose plus 1 per cent. dextrose, the mats were of equal size and about twice the diameter of those on 2 per cent. galactose (table II). On agar, without sugar, the mats were of very meager thickness, but in all cases extended as far or farther than those on 2 per cent. dextrose (table III). Fruiting branches were very rare on the

[1] This does not give an exact measure of the amount of acceleration, as there is a greater area for the growth of the organism in the presence of the more usable sugar in the mixed solutions.

TABLE II

GROWTH OF *ASPERGILLUS NIGER*, DIAMETER OF MATS*

SUGAR	HOURS				
	52	76	100	124	148
	mm.	*mm.*	*mm.*	*mm.*	*mm.*
2% galactose	4.95	6.65	6.65	11.2	16.1
2% dextrose	8.6	13.25	20.2	26.5	34.45
1% galactose plus 1% dextrose	8.0	12.62	19.62	26.75	34.75

* Each measurement is the average for five culture dishes.

medium which contained no sugar. It was apparent that there was a great difference in the thickness and density of the mats formed on the different sugars. These plates were inoculated with a small drop of spore suspension.

An attempt was made to correlate the density of the growth with the linear development of the mycelium. Agar petri dish cultures were again used and the total extent of the mat measured with a low power microscope. When no sugar was supplied there were again extensive but very meager mats formed, with no upright mycelium produced in five days. When galactose was used alone in the medium, the diameter of the mat was inversely proportional to the amount of sugar. When the sugars were mixed there was in both cases an acceleration. The relative thicknesses of the mats were estimated in order, without attempting an accurate measurement. Thus the figures in table IV give only relative thicknesses.

TABLE III

GROWTH OF *PENICILLIUM GLAUCUM*, DIAMETER OF MATS, PETRI DISH AGAR CULTURES, 20° C.

SUGAR	HOURS				
	52	76	100	124	148
	mm.	*mm.*	*mm.*	*mm.*	*mm.*
2% galactose	5.95	9.10	12.15	15.15	17.95
2% dextrose	8.5	14.94	22.	29.12	35.19
1% galactose plus 1% dextrose	8.69	15.44	22.	28.75	35.12

The results given in table V show the growth rates of *Aspergillus niger* on galactose and dextrose and mixtures of these two sugars for 65 hours on agar coverglass cultures. In this test the germination of the spores is greatly delayed on the agar containing only galactose. There is also an abnormal growth on the galactose medium, as indicated by the crooked and much-

TABLE IV

GROWTH OF *ASPERGILLUS NIGER*, AGAR CULTURES AT 20° C. FOR FIVE DAYS

SUGAR	ORDER OF SPORULATION	DIAMETER OF MATS	THICKNESS*
		mm.	
2% galactose	5	2	7
1.8% galactose	9	14	8
1.0% galactose	7	20	6
1.0% galactose plus 1.0% dextrose	1	38	1
1.8% galactose plus 0.2% dextrose	4	35	4
0.2% dextrose	6	31	5
1.0% dextrose	3	34	3
2.0% dextrose	2	34	2
No sugar	8	30	9

* Order of relative thickness. Eight and nine, column 2, were practically equal, as were six and seven of column 4.

TABLE V

GERMINATION PERCENTAGE AND GROWTH (MM.) OF *ASPERGILLUS NIGER*, AGAR COVERGLASS CULTURES, 28° C.

SUGAR	HOURS						
	4	8	12	16	24	30	65
	%						
2.0 galactose	0	0	0	0	25% 0.0375	90%†	95%
1.8 galactose plus 0.2 dextrose	50% 0.07	0.15	0.43	1.20‡	.....		
1.0 galactose plus 1.0 dextrose	50% 0.114	0.16	0.90				
1.0 galactose	0	0		5% 0.007*	15% 0.065	60%† 0.06	90% 0.10
0.2 dextrose	70% 0.0383	0.074	0.594	0.9‡	.....		
1.0 dextrose	65% 0.027	0.0825	0.465	0.67‡	.....		
2.0 dextrose	50% 0.025	0.1007	0.39	0.75‡	.....		

* Longest germ tube in the culture.

† Crooked, much branched mycelium.

‡ Increased germination shortened the average length of the germ tubes. Stopping of the measurements indicates that the mycelium was too long to be measured accurately.

branched mycelium shown in figure 6, as compared with figures 7 and 8. The growth on the dextrose and the dextrose plus galactose varies consid-

FIG. 7. *A. niger* grown 16 hours at 28° C. on 2% dextrose.

erably, with the greatest growth on the medium containing 1 per cent. of each sugar.

In mixing galactose and levulose, the growth rate is accelerated much as it is with galactose and dextrose. When mannose (which used alone as a carbohydrate supply also gives a very slight growth) was mixed with galactose there also resulted an acceleration comparable with that obtained with galactose and dextrose, or galactose and levulose. Levulose mixed with mannose, however, gave only a slight increase in growth over that obtained when levulose was used alone. When mannose was added to the solution containing galactose and levulose there was apparently no additional acceleration.

The dry weights of the mycelium mats in grams are given in figure 9, in

FIG. 8. *A. niger* grown 14 hours at 28° C. on 1% galactose and 1% dextrose.

which the area of each circle indicates the relative amount of dry weight produced.

The growth rates of *Aspergillus niger* and *Penicillium glaucum* on lactose and sucrose, pure and in mixtures, were investigated. In the curves of figures 10 and 11 there is no acceleration due to the presence of these two sugars in one culture solution. Reducing the amount of sucrose present does not greatly reduce the growth of the organisms, for the growth periods used, until the sucrose is reduced to 1 per cent. or less. There is then a more rapid drop in the amount of growth to a very low point on the 2 per cent. lactose solution.

A correlation was made between the dry matter formed and the increases in acidity during 48-hour periods. These preliminary experiments indicate that pure galactose produces the greatest acidity per gram of dry matter

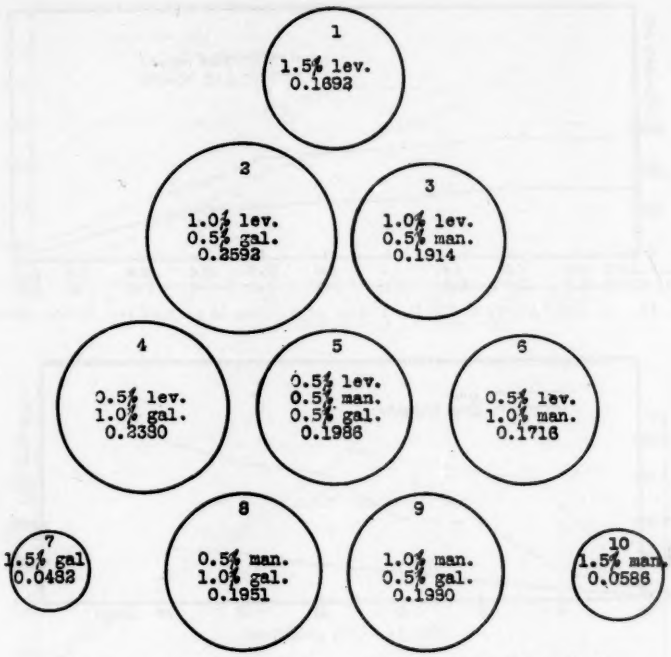

FIG. 9. *A. niger* grown 14 days at 20° C. in three 150-cc. flasks.

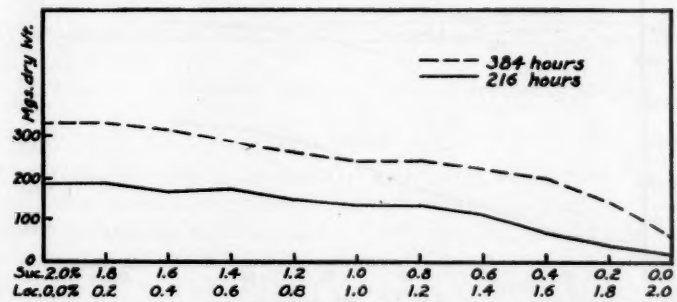

FIG. 10. *P. glaucum* grown at 20° C.; 100 cc. of solution in each of two 125-cc. flasks.

formed, galactose plus dextrose produces the least, and pure dextrose is intermediate in the production of acid. The total acidity in the pure galactose is less than that produced on the pure dextrose or on the dextrose plus galactose, indicating that it must be within the range for favorable growth of these organisms (figs. 12–14).

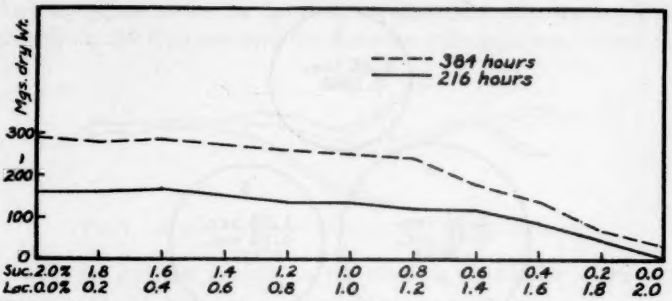

FIG. 11. *A. niger* grown at 20° C.; 100 cc. of solution in each of two 125-cc. flasks.

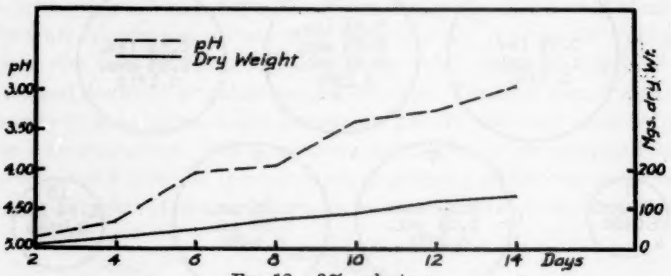

FIG. 12. 2% galactose.

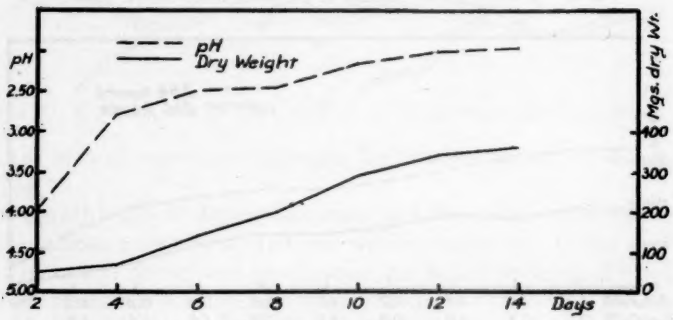

FIG. 13. 2% dextrose.

Comparisons were made with cultures grown on solutions sterilized in the Arnold steam sterilizer and those grown on cultures sterilized by filtering through porcelain filters. The data presented in figure 15 eliminate the possibility of there being any significant changes in the mixed sugars during the process of heat sterilization.

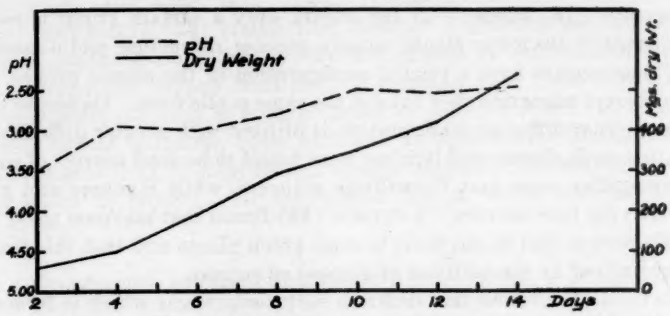

FIG. 14. 1% galactose and 1% dextrose.

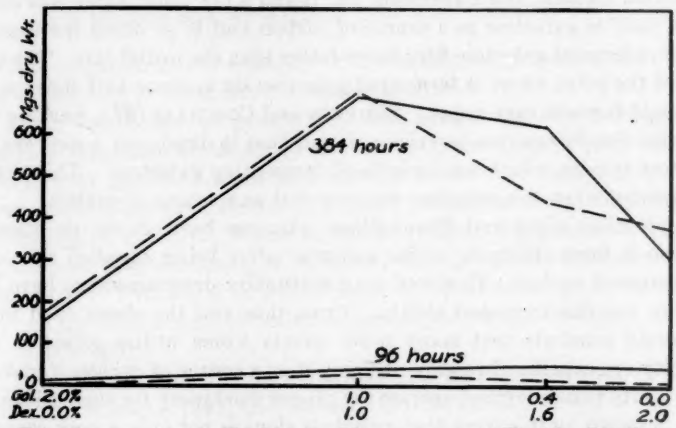

FIG. 15. ——— sterilized in Arnold; — — — sterilized by filtration.

Discussion

The frequent occurrence in plants of compounds which upon hydrolysis yield galactose would tend to imply that that sugar must play some part in the normal metabolism of those plants. LIPPMAN (**16**) found a thin coat of galactose on the surface of frozen ivy fruits. It is not positively known that this did not result from hydrolysis of a glucoside. CZAPEK (**5**) states that it is questionable whether galactose ever occurs free in plants.

Among the compounds which upon hydrolysis yield galactose are glucosides and galactans. The first named are probably split up by enzymes, but GORTNER (**8**) states that "insofar as is known, there are no known specific enzymes which will hydrolyze galactans." On the other hand, HAAS and HILL (**9**) state that "the galactans found in the cotyledons of many plants are used as reserve foods."

MAXIMOV (**18**) states: "Of the sugars, only a certain group is readily transformed by the lower plants, namely, glucose, d-fructose, and d-mannose. All of these sugars have a related configuration of the atomic groups, and, with hydroxyl migration they exhibit the same enolic form. Galactose showing a somewhat different configuration is utilized with greater difficulty."

In this work glucose and levulose were found to be good sources of carbon for *Aspergillus niger* and *Penicillium glaucum*, while mannose and galactose were very poor sources. KNUDSON (**13**) found that mannose acted more like galactose in that it was toxic to some green plants and that this toxicity was neutralized by the addition of glucose or sucrose.

KENDALL (**11**) found that dextrose is the only sugar which is fermented by all sugar-fermenting organisms.

EULER, LAURIN, and PATTERSON (**6**) found a top yeast which was able to adapt itself to galactose as a source of carbon and in so doing increased its ability to ferment galactose fifty times faster than the initial rate. The yeast reached the point where it fermented galactose six and one-half times as fast as it could ferment cane sugar. SÖHNGEN and COOLHAAS (**27**), working with the yeast *Saccharomyces cerevisiae*, found that it developed a new enzyme, galactose-zymase, which was capable of fermenting galactose. This enzyme disappeared when the galactose was removed as a source of carbon.

Aspergillus niger and *Pennicillium glaucum* have shown an apparent increase in their ability to utilize galactose after being supplied this sugar as a source of carbon. However, no quantitative determinations have been made to test this increased ability. From this, and the above cited works, one would conclude that many lower plants which utilize galactose with difficulty are capable of readily utilizing it as a source of carbon if given an opportunity to develop and operate the proper machinery for such utilization.

It is shown by this work that galactose alone is not only a poor source of carbon for *A. niger*, but that it slows up spore germination, retards growth of the mycelium, and also causes abnormal growth. This is shown in table V where there are visible signs of germination of *A. niger* spores in 16 hours on 1 per cent. galactose, but 24 hours were required on a 2 per cent. galactose solution. On dextrose, dextrose plus galactose, and on agar alone there was at least 50 per cent. germination within the first four hours. When the same mineral nutrients and agar without sugar are supplied we find the development of mycelium threads much longer than when galactose has been added, thus indicating that galactose under these conditions actually retards the growth of *A. niger* on a medium where it would normally produce a limited amount of mycelium. Figure 6 shows that on agar containing only galactose as a source of carbon the mycelium threads are much branched and very crooked as compared with those on 1 per cent. dextrose or 1 per cent. dextrose plus 1 per cent. galactose, as is shown in figures 7 and 8.

KUNKEL (**15**) states that toxicity retards germination and growth of fungi. SCHELLING (**25**) claims that vitamin B preparations stimulate *A. niger* to abnormal development like that caused by toxins, namely, short, blunt, irregularly shaped branches. WATERMAN (**31**) finds that mutation of *Penicillium glaucum* is brought about in cultures which retard growth. He lists lactose, galactose, boric acid, and all narcotics as substances which check the growth of *P. glaucum* and cause mutation. This mutation appears after about 14 months. He also found that *A. niger* mutates from the effects of:

A. Poisons such as copper sulphate and benzoic acid.
B. Narcotics such as paraoxybenzoic acid and salicylic acid.
C. (1) Culture media with galactose or polysaccharides containing the galactose group as a carbon source; (2) culture media with glutaric acid, tartaric acid, antitartaric acid, and rhamnose.

This mutation consists largely of a checking of growth, a loss of color, and a reduction in the number of spores produced.

If the retardation of germination and growth and the development of abnormal mycelium are accepted criteria for toxicity, then, according to the present data, galactose when used alone as a source of carbon is toxic to *A. niger* and *P. glaucum*. However, I would hesitate to accept these results as indications of toxicity. Would it not be possible for galactose to act as a growth retarding substance, on *A. niger* and *P. glaucum*, without necessarily being toxic? This might be true of other so-called toxins which do not cause any actual destruction of cells, as was reported by KNUDSON (**12**) and HEINONEN (**10**).

It is evident from the data of BOAS and MERKENSCHLAGER (**1**), BURGE *et al.* (**3**), ROBBINS (**24**), and ROACH (**23**) that galactose is not toxic to all green plants. Also, the literature indicates that some non-green plants are able to utilize galactose as well as or even better than they can other sugars.

In the work of KNUDSON (**12**) and HEINONEN (**10**) it was found that the addition of some other sugars neutralized the toxicity of galactose toward the green plants they used. In my work it is found that when dextrose, levulose, or mannose is added to galactose, there is a decided acceleration in the growth of *A. niger* and *P. glaucum*, as indicated by the weight of dry matter formed. This is similar to the results of KNUDSON (**14**), who found that when timothy seedlings were grown on a medium containing lactose and sucrose there was a decided acceleration in growth rate. When KNUDSON mixed galactose and mannose, both of which he found to be toxic to the plants he worked with, there was no neutralization of the toxicity; whereas in my work there was a decided acceleration in growth when these two were mixed. The degree of this acceleration is determined by the percentage of

each sugar in the individual mixtures used and is apparently modified by other factors. In each set of culture flasks set up as in figures 1, 2, and 3, there has always been two optimum solutions. These remain relatively constant for the series set up from a given stock solution, but there are some variations between the sets set up from different stock solutions. The cause of this variation has not been determined. Probably if the average were taken from enough sets of cultures made up from different stock solutions, there would be only one growth peak which would cover several of the mixtures.

This acceleration in growth must be due to the presence of the galactose, since there is no appreciable acceleration when mannose and levulose are mixed as in numbers 3 and 6, figure 9. This is also true in figures 10 and 11. The last two examples correspond to the work of SOBOTKA and REINER (**26**), in which they report that the rate of fermentation of mixed sugars is barely greater than that of the fastest fermenting component. This cannot be the case when galactose is mixed with glucose, levulose, or mannose and used as a source of carbon for *A. niger* and *P. glaucum* because of the acceleration obtained from the mixtures of sugars.

This acceleration in growth may produce three times as much dry weight on two sugars mixed as the sum of the dry material produced on the same sugars used separately, the total amount of sugars and growing surfaces being equal in each case. This is seen in comparing the total weight produced by numbers 2 and 5 with number 6, and that of 3 and 4 with number 7, of table I.

Table V shows that during the early growth period, the first 16 hours, there is an acceleration in the rate of elongation of the mycelium when there is galactose mixed with dextrose, over that on either of the sugars alone.

If one is to accept MAXIMOV'S (**19**) statement that "of the great variety of organic compounds of different degree of availability plants will always utilize the more available compounds and only then begin to use the less assimilable ones," this growth then must all be at the expense of the dextrose, mannose, or levulose present in the mixed solutions. If this is true, then we find galactose acting exactly opposite from what it does when used alone. KNUDSON (**14**) suggested that there might be a polymerization of the galactose and dextrose to form lactose and thus neutralize the toxic action of galactose. Referring to figures 10 and 11, it is seen that such could not be the case here because of the very small amount of growth on lactose. KNUDSON also suggested that dextrose might prevent the absorption of galactose. That would not apply to this work, because with the addition of galactose to dextrose, levulose, or mannose there is an acceleration. This acceleration surely would not occur unless the galactose in some way came into contact with the protoplasm of the growing cells. Another suggestion by KNUDSON (**12**) is

that the oxidation products of galactose may be toxic to green plants. This is in accord with the idea of FUNKE (7) that galactose and mannose do not prevent enzyme production but that their metabolic products do. This, too, probably would not apply to the results here presented because of the inhibition of germination and growth from the time of inoculation.

There is no clue to this growth acceleration found in figures 12, 13, and 14, where the dry weight and the increase in acidity are compared.

This leaves unsolved the question as to the action of any one sugar when it neutralizes the toxicity of another sugar, or when galactose causes an acceleration in the growth of *A. niger* and *P. glaucum* when it is mixed with dextrose, levulose, or mannose in the culture solution.

Summary

1. Galactose and mannose are poor sources of carbon for *Aspergillus niger* and *Penicillium glaucum*.

2. Galactose, when used alone as a source of carbon, decreases the rate of spore germination and mycelium development and causes abnormal mycelium development.

3. Galactose, when mixed with dextrose, levulose, or mannose, causes an acceleration in growth. This is not caused by mannose, which is also a poor source of carbon for these organisms.

4. The addition of lactose to a culture solution containing sucrose has little if any effect upon the growth rate.

5. Preliminary correlations on the amount of dry matter formed and the acidity developed do not indicate that the acidity of the solution plays any important part in the acceleration of growth.

6. The data hold for the strains of *A. niger* and *P. glaucum* used; other organisms or other strains of these organisms may react entirely differently.

The writer wishes to express his appreciation to Dr. CHARLES A. SHULL for the many helpful suggestions and criticisms offered throughout the progress of this work. He is also grateful to The Elizabeth Thompson Science Fund for a grant for the purchase of sugars, and to The Graduate Research Fund of the University of Kansas for grants for the purchase of apparatus and supplies.

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EFFECT OF PETROLEUM OILS ON THE RESPIRATION OF BEAN PLANTS, APPLE TWIGS AND LEAVES, AND BARLEY SEEDLINGS[1]

JESSE R. GREEN

(WITH TWO FIGURES)

Introduction

This study of the effect of petroleum oils on the respiration of plants is part of an investigation to determine the cause of injury to plants by oil sprays. In an earlier paper (**3**) it was reported that oils containing more than 15 per cent. sulphonatable residue caused an increase in respiration over the normal rate, and oils with less than that amount caused a decrease. It was stated that this relation was not true in every case, but was the average of all the determinations made at that time. The results varied considerably and it was believed that by improving the methods of measuring carbon dioxide, a more definite relation could be established between the toxicity of an oil and its effect on respiration.

Materials

OILS.—Commercial spray oils, the properties of which are given in table I, were used in this investigation. Inasmuch as the sulphonatable materials

TABLE I

PROPERTIES OF OILS USED ON PLANTS IN RESPIRATION STUDIES

LAB. NUMBER	SULPHONATABLE PORTION	VISCOSITY (SAYBOLT AT 100° F.)	SPECIFIC GRAVITY	INJURY RATING*
	%	HIGHLY REFINED OILS		
3	6.0	113	0.873	3
20	1.0	98	0.879	4
22	2.0	59	0.863	2
24	None	68	0.837	1 (least injurious)
		POORLY REFINED OILS		
5	41.6	136	0.919	6
13	31.2	82	0.908	8
15	26.4	62	0.896	9 (most injurious)
21	15.6	56	0.884	5
28	35.2	110	0.923	7

* See (2) for calculation.

[1] Paper no. 60 of the Journal Series of Montana Agricultural Experiment Station. Contribution from the Department of Chemistry.

in an oil have considerable effect on respiration, the oils were selected on the basis of the sulphonatable materials which they contained. These materials are composed of unsaturated hydrocarbons, naphthenic acids, and other compounds containing aromatic groups, sulphur, and nitrogen. They are removed, either partly or completely, in the refining process by treatment with sulphuric acid. For convenience in the present work, oils containing not more than 15 per cent. of sulphonatable material will be considered as highly refined oils and those containing more than this amount will be considered as poorly refined oils.

In a previous investigation (**2**) it was shown that oil no. 24, a highly refined oil, was the least injurious to plants and that oil no. 15 was the most injurious. As these two oils represented the extremes in the two classes, they were used in most of these determinations.

PLANTS.—The tops of bean plants, apple twigs and leaves, and barley seedlings were used in the respiration studies. Young bean plants 8–12 inches high were cut off at ground level and the stems placed in water, three plants being used in each sample. The samples of apple twigs bearing the leaves that were to be sprayed in the laboratory consisted of three twigs about 12 inches long and were all cut from the same side of one tree for each determination. The apple twigs and leaves that had been sprayed in the orchard were taken from trees on which some limbs had been sprayed and others left for checks. In this case, however, the control and sprayed samples were taken as closely together as possible. Barley seedlings were grown in small jars in quartz sand with nutrient solutions, about 40 plants being grown in each jar. The jars containing the plants were placed directly in the respiration chambers and the respiration was thus measured under very nearly natural conditions.

Method

SPRAYING.—In the laboratory the oils were applied to the plants with an atomizer, the plants being rotated during the process to insure a uniform distribution of the oil. The atomizer used in these studies was similar to that described before (**3**) and was calibrated to deliver the same amount of oil on each sample. The calibration was done by spraying oil on an 18.5-cm. filter paper for one minute. The number of seconds to spray 0.1 gm. of oil on the filter paper was calculated from the weight of oil sprayed in one minute and all the plants were sprayed for this length of time. One-tenth gm. of oil on a circular area 18.5 cm. in diameter is equivalent to 0.0037 gm. per square cm. This amount of oil makes a film which is easily seen on the plants. The apple twigs and leaves sprayed in the field were sprayed with regular field equipment and with the field emulsions recommended in practice (**7**).

RESPIRATION MEASUREMENTS.—Respiration in plants is measured in terms of the interchange of gases brought about by the metabolic process. All the gases of the atmosphere are involved and especially carbon dioxide and oxygen. In this work carbon dioxide measurements only were considered.

Sixty-two determinations were made with the improved respiration apparatus already described (**6**). In each determination six samples were used, three were sprayed with the oil being investigated and three were used as controls. Measurements of the output of carbon dioxide by the plants were made at two-hour intervals for eight hours or longer. The average amount of carbon dioxide produced by the three controls was considered the normal rate and compared with the average of the three sprayed samples. The difference was computed to percentage gain or loss from the normal rate.

All plants, excepting those used in two series described later, were kept in darkness at a constant temperature of 28° C., and the carbon dioxide respired was absorbed in barium hydroxide and subsequently determined by titrating with hydrochloric acid. MILLIGAN absorbers (**8**) were first used and later a bead absorber was designed, but neither gave complete absorption of carbon dioxide when low concentrations of barium hydroxide were used. Finally a new spiral absorber (**6**) was designed which proved highly efficient even with very dilute absorbing solutions.

Results

BEAN PLANTS.—The effect of the sulphonatable materials in the two classes of oils was studied throughout the work even though some other

TABLE II

EFFECT OF OILS ON THE RESPIRATION OF BEAN PLANTS

OIL NO.	GAIN IN RESPIRATION
	HIGHLY REFINED OILS %
3	0.4
20	10.7
24	3.5
22	12.8
Total	27.4
Average gain	6.8
	POORLY REFINED OILS %
5	28.5
28	28.0
21	4.6
13	29.5
Total	90.6
Average gain	22.6

variable was being especially considered. Table II shows the results of four determinations made by using highly refined oils on bean plants in which the average respiration was increased 6.8 per cent. Four other determinations, made with poorly refined oils, caused a much greater increase, 22.6 per cent.

APPLE TWIGS AND LEAVES.—Six determinations (table III) with apple twigs sprayed in the field with emulsions of highly refined oil no. 24 caused

TABLE III

RESPIRATION OF APPLE TWIGS AND LEAVES SPRAYED IN THE ORCHARD WITH OIL EMULSIONS

EMULSIFIER	CONCENTRATION OF OIL	DATE OF SPRAYING	DATE OF ANALYSES	TIME ELAPSED	RESPIRATION	
					GAIN	LOSS
	%			*days*	%	%
	OIL NO. 24, SULPHONATABLE PORTION, NONE					
Ammonium casenate	8	June 6	June 10	4		18.4
Ammonium casenate	8	June 6	June 17	11		42.8
Ammonium casenate	8	Aug. 13	Aug. 18	5		4.8
Cresoap	8	Aug. 13	Sept. 21	39		13.1
Ammonium casenate	4	June 6	Sept. 20	106	30.3	
Cresoap	4	Aug. 13	Aug. 25	12	3.9	
			Total		34.2	79.1
			Average loss			7.5
	OIL NO. 13, SULPHONATABLE PORTION, 18.3 PER CENT.					
Ammonium casenate	8	June 6	June 12	6	27.5	
Ammonium casenate	8	Aug. 13	Aug. 19	6	22.9	
Cresoap	8	Aug. 13	Aug. 20	7	67.7	
Cresoap	4	Aug. 13	Aug. 26	13	12.7	
Cresoap	8	Aug. 13	Sept. 26	13	29.6	
Cresoap	8	May 27	Sept. 25	122	22.3	
			Total		182.7	
			Average gain		30.4	
	OIL NO. 5, DORMANT SPRAY					
Cresoap	16	April 17	May 12	25	40.0	

respiration to be retarded 7.5 per cent. Six other determinations on samples sprayed with emulsions of poorly refined oil no. 13 showed that on the average respiration was increased 30.4 per cent. Furthermore, this effect extends over long periods, as one sample of twigs sprayed in the early bud stage with poorly refined oil no. 15 showed an increased respiration of 40 per cent. 25 days after spraying.

Apple twigs and leaves sprayed in the laboratory with unemulsified oil no. 15 did not show a significant change in the rate of respiration. The

seven determinations, as shown by the data in table IV, made an average gain of 2.2 per cent. in respiration. However, this work was done in Sep-

TABLE IV

EFFECT OF OILS ON RESPIRATION OF APPLE TWIGS AND LEAVES SPRAYED IN THE LABORATORY WITH POORLY REFINED OIL NO. 15

DATE OF SPRAYING	CHARACTER OF DAY PRECEDING DETERMINATION	RESPIRATION	
		GAIN	LOSS
		%	%
Sept. 26	Clear and warm	.....	4.4
Sept. 28	Clear and warm	5.5	.....
Sept. 30	Clear and warm	.....	3.3
Oct. 1	Clear and warm	.....	1.0
Oct. 2	Clear and warm	10.5	.....
Oct. 3	Clear, minimum temperature of 26° the night before	.....	2.1
Oct. 6	Clear, minimum temperature of 33° the night before	10.3	.....
	Total	26.3	10.8
	Average gain	2.2	.....

tember and October after light frosts, and the leaves were not very responsive. It has been observed on many occasions that young tender leaves respond readily to factors that influence respiration. Fully developed leaves obtained in the fall as used in the foregoing experiments should not be expected to show much effect of oil.

RESPIRATION OF BARLEY SEEDLINGS.—Thirty-four determinations with barley seedlings were made. On account of the apparent irregularity in the respiration of plants, efforts were made to determine some of the factors affecting respiration. Age of plants was thought to be an important factor. There was also the problem of drought injury, which easily occurs when plants are grown in sand cultures. The quantity of light preceding the determination and the amount of oil applied to the plants were investigated.

AGE OF PLANTS.—The effect of oil on barley seedlings of different ages is shown in table V. Four determinations were made with seedlings ranging from 8 to 18 days old sprayed with poorly refined oil no. 5, and an average gain in respiration of 35.5 per cent. was found. Four other determinations were made with highly refined oil no. 24 sprayed on plants from 7 to 13 days old with an average gain in respiration of 1.8 per cent. Several months later similar series of determinations were made, using poorly refined oil no. 15 on plants ranging from 7 to 17 days old and the rate of respiration was again found to be increased by 8.6 per cent. Highly refined oil no. 24, used in four other determinations in which the plants were from

TABLE V

EFFECT OF SPRAYING POORLY AND HIGHLY REFINED OILS ON RESPIRATION OF BARLEY SEEDLINGS OF DIFFERENT AGES

Series	Age of plants	Respiration		Age of plants	Respiration	
		Gain	Loss		Gain	Loss
	days	%	%	*days*	%	%
	Poorly refined oil no. 5			Highly refined oil no. 24		
First	8	8.2		7	4.8	
	10	22.9		9		7.1
	16	50.3		11	8.1	
	18	56.1		13	1.8	
	Total	137.5		Total	14.7	7.1
	Average gain	34.9		Average gain	1.9	
	Poorly refined oil no. 15			Highly refined oil no. 24		
Second	7	4.9		8	6.0	
	11	7.5		12		3.4
	14	25.4		19	16.0	
	17		5.5	26		5.4
	Total	37.8	5.5	Total	22.0	8.8
	Average gain	8.1		Average gain	3.3	

8 to 26 days old, increased the respiration only 3.3 per cent. It will be seen from these data that, regardless of the difference in age of the barley seedlings, poorly refined oils increase respiration more than highly refined oils.

DROUGHT.—The effects of drought injury are shown to be very erratic (table VI). The indications are that highly refined oil no. 24 causes a much greater increase in the rate of respiration on plants that were suffering, or that had suffered, drought injuries than on normal plants. The four determinations with plants sprayed with poorly refined oils nos. 13 and 15 indicate a considerable increase in the respiration rate of drought injured plants so sprayed. These results, however, are not comparable with the plants sprayed with oil no. 24. The effect of drought on the plants before and during respiration measurements cannot be stated as a result of these limited experiments.

LIGHT.—The relation of light to the respiration of plants sprayed with oil was not studied extensively. In one test the plants were kept in darkness for two nights and a day before spraying, in order to show an extreme effect of the lack of light. Oil no. 24 was used and a loss in the respiration rate of 3.3 per cent. was found. Another determination, made with the same oil and the same kind of plants, except that the plants were well lighted with artificial and natural light for the day and night previous to

TABLE VI

EFFECT OF OIL ON BARLEY SEEDLINGS SUFFERING FROM DROUGHT INJURY

Oil no.	Respiration		Remarks
	Gain	Loss	
24	.....	6.8	No drought injury
24	38.5	.....	Plants wilted day before determination was made, but well watered and turgid during determination
24	2.4	.....	No drought injury
24	43.1	.....	Plants wilted day before determination was made, but well watered and turgid during determination
15	11.3	.....	No drought injury
15	25.7	.....	Plants just at wilting stage when determination was made
13	.....	22.2*	No drought injury
13	.....	1.6	Plants wilted day before determination was made, but well watered and turgid during determination
13	16.1	.....	Plants just at wilting stage when determination was made
13	20.5	.....	Plants wilted
5	.....	9.3*	No drought injury
5	.....	26.0	Plants wilted day before determination was made, but well watered and turgid during determination

* With poorly refined oils 5 and 13 an increase in respiration was to be expected, but in these cases a considerable decrease was obtained.

making the respiration measurements, showed a gain of 10.3 per cent. as a result of spraying. In the test following a period of darkness, which presumably had lowered the carbohydrate content, respiration was decreased by spraying with a highly refined oil. The carbohydrates apparently were not decomposed to form CO_2 in the sprayed as fast as in the normal plants. In the second test following a long period of light, the carbohydrates should

have been built up in the plants. Under such conditions spraying with a highly refined oil caused an increase in the rate of carbohydrate decomposition and consequently greater production of carbon dioxide, or a greater rate of respiration. Determinations with poorly refined oils were not made under comparable conditions.

AMOUNT OF OIL.—The effect of the amount of oil applied was also tested. Oil no. 15 was sprayed lightly on the barley seedlings and a gain of 11.3 per cent. in the rate of respiration was found. In another test the same oil was sprayed on the same kind of plants until they were dripping and a gain of 10.3 per cent. was found, which is but little different from that obtained when only a thin film of oil covered the plant.

Another determination was made with six samples of barley seedlings. The first sample was not sprayed at all and the others received increasing amounts. The results are given in table VII. The single control sample

TABLE VII

EFFECT OF INCREASING AMOUNTS OF OIL NO. 15 (POORLY REFINED) ON RESPIRATION OF BARLEY SEEDLINGS

	SAMPLE NO.					
	1	2	3	4	5	6
Number of minutes plants sprayed with oil	0	½	1	1½	2	2½
CO_2 respired per plant in 8 hours (mg.)	1.20	1.17	1.12	1.09	1.13	1.08

had a higher rate of respiration than the others, but conclusions cannot be drawn from one control. The results indicate, however, that increasing amounts of oil do not change the rate of respiration.

RESPIRATION OVER LONG PERIODS.—A determination of 80 hours' duration was made with barley seedlings sprayed with oil no. 15 in order to show the influence of oil on respiration over long periods. For the first 48 hours

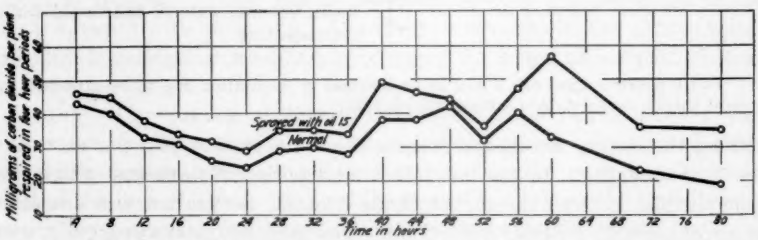

FIG. 1. Rate of respiration of normal and oil sprayed barley seedlings over a period of 80 hours.

the plants were illuminated with artificial light and for the rest of the time they were kept in darkness. Figure 1 and table VIII show the results of

TABLE VIII

EFFECT OF OIL NO. 15 ON RESPIRATION OF BARLEY SEEDLINGS FOR PERIOD OF 80 HOURS

TIME	CO_2 GIVEN OFF BY EACH CONTROL PLANT IN 4 HOURS	CO_2 GIVEN OFF BY EACH PLANT SPRAYED WITH OIL NO. 15 IN 4 HOURS
hours	*mg.*	*mg.*
4	0.43	0.46
8	0.40	0.45
12	0.33	0.38
16	0.31	0.34
20	0.26	0.32
24	0.24	0.29
28	0.29	0.35
32	0.30	0.35
36	0.28	0.34
40	0.38	0.49
44	0.38	0.46
48	0.42	0.44
52	0.32	0.36
56	0.40	0.47
60	0.33	0.57
70*	0.57	0.66
80*	0.49	0.62
Total	6.13	7.35
Increase in respiration caused by oil = 19.9%		

* Values obtained at 10 hour intervals have been computed to value for 4-hour periods and plotted in figure 1.

this determination. The abrupt turn downward of the respiration curves after 48 hours is due to a slight drop in temperature resulting from the lights being turned off. Good temperature control could not be obtained because of the heat from the powerful lights, but there was no crossing of the curves at any time. The respiration of the sprayed plants was always above that of the normal plants and during the 80 hours they produced 19.9 per cent. more carbon dioxide than did the normal plants.

Another determination of 36 hours' duration (table IX) was made with barley seedlings sprayed with oil no. 24. The plants were artificially lighted for the entire period. The curves in figure 2 show that the sprayed plants respired at a greater rate throughout the whole period than did the normal plants. The total increase in respiration of the sprayed plants was 16.1 per cent.

TABLE IX

EFFECT OF OIL NO. 24 ON RESPIRATION OF BARLEY SEEDLINGS FOR PERIOD OF 36 HOURS

TIME	CO_2 GIVEN OFF BY EACH CONTROL PLANT IN 4 HOURS	CO_2 GIVEN OFF BY EACH PLANT SPRAYED WITH OIL NO. 24 IN 4 HOURS
hours	*mg.*	*mg.*
4	0.39	0.41
8	0.59	0.57
12	0.54	0.59
16	0.47	0.55
20	0.40	0.49
24	0.38	0.50
28	0.39	0.50
32	0.39	0.49
36	0.39	0.48
Total	3.94	4.58
Increase in respiration caused by oil = 16.1%		

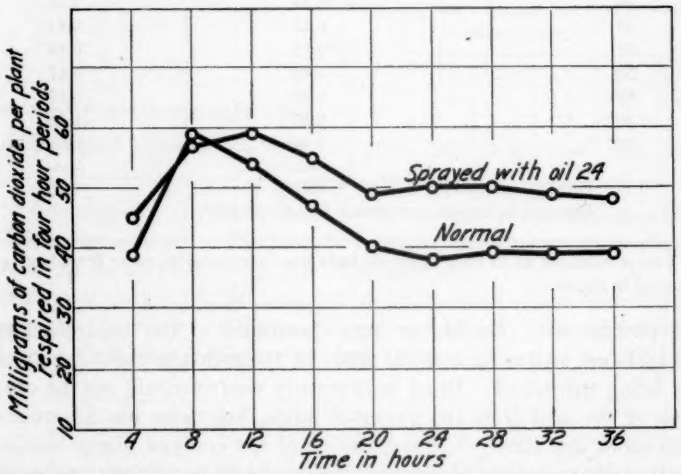

FIG. 2. Rate of respiration of normal and oil sprayed barley seedlings over a period of 36 hours.

In order to show further the continued effect of oil on respiration, a determination was made in the usual way with oil no. 15. A loss of 1.2 per cent. in the rate of respiration was found. The plants were taken from the respiration apparatus and kept under normal conditions for two days, after which their rate of respiration was again measured and showed a loss of

20.7 per cent. This is only one of the cases in which a poorly refined oil, which usually increases respiration, has caused a decrease. Also the retardation of respiration was found on the same plants at two different times.

The foregoing experiments indicate again that changes in the respiration rate caused by oil are long continued and that probably there is not a periodic rise and fall in the rate as might be expected. In plotting the data from several 8-hour experiments, the changes in the respiration rate caused by oil do not rise above nor fall below the normal, but rise and fall the same as the normal rate.

The average gain in respiration in 17 determinations made on barley seedling samples sprayed with poorly refined oils was 12.0 per cent. In 13 determinations made on barley seedlings sprayed with highly refined oils the average respiration gain was 8.8 per cent. In 35 determinations made with the various plants sprayed with poorly refined oils the average gain was 15.6 per cent., and in 23 determinations made with various plants sprayed with highly refined oils the average gain in respiration was 4.2 per cent.

Discussion

Measurements of plant respiration are difficult to interpret. It is evident from the data that some variables are not controlled. Poorly refined oils generally caused a greater increase in the respiration rate than did the more highly refined oils. Just why either class of oil sometimes causes a decrease in the rate of respiration is not yet known, however. A determination can be made on one day with a given oil and an increase in the rate will be shown. A similar determination may be made on the next day, with conditions apparently identical, and with plants that were grown beside those that were used on the previous day, and a loss in the rate of respiration may be shown. No reasons have been found to explain these facts.

Although respiration has been extensively investigated, there is still much uncertainty regarding the fundamental reactions involved. The complexity of the chemistry of respiration is admitted. When considering the process from the standpoint of the production of carbon dioxide, it is reasonable to assume that the last step in the reactions involved is the oxidation of organic compounds. On the other hand, the taking up of carbon dioxide must then be followed by the reduction of this substance. The terms oxidation and reduction are here used in their older sense, in which oxidation is considered the addition of oxygen and reduction is the subtraction of oxygen. The modern conception of the two terms, or the idea of the change in valence, will also hold true. Both reduction and oxidation are in all probability going on at the same time in a plant. Speaking of increased respiration, or greater evolution of carbon dioxide, simply means that oxi-

dation is favored. When smaller amounts are given off, reduction has been favored.

The situation cannot be shown as a simple reversible reaction, but the complete set of reactions are subject to the same laws of mass action; that is, the abundance of the component on the two sides of the equation determines the direction in which the action will take place. It is fair to assume that if carbohydrates are accumulated on one side, the reaction will tend to move in the direction of oxidation and the production of carbon dioxide. If carbohydrates are low in the plant, the reverse action is favored and carbon dioxide is absorbed at a greater rate.

Energy relations are equally important in influencing the direction of the reacting substances. It is well known that light favors the taking up of carbon dioxide and thus, according to the theory proposed, brings about reduction. Inasmuch as the energy of biological reactions is controlled largely by the action of enzymes, it is entirely possible that the failure to get consistent changes in the rate of respiration with a given kind of oil was due to a lack of knowledge of these organic catalysts. POPE (**9**) found that catalase activity was inversely proportional to carbon dioxide production in young barley seedlings. Films of oil over the surface of plant cells undoubtedly have some influence on the enzyme catalase and in this way change the rate of respiration.

The theory so often proposed, that oil films close the stomatal opening, is not favored by these tests because the small amounts of oil sprayed on the plants produced about the same change in respiration rate as did the large amounts. It may be some toxic effect of a fraction of the petroleum oil which really causes the damage to plants, and any interference by the oil to the flow of gases in the plant is of minor importance. Some investigations (**5, 1, 4**) have been made to determine the cause of injuries by petroleum products to men working in the oil industry. There is a probability that the portion of petroleum which is toxic to man may also be injurious to plants.

Summary

1. The averages of several tests with plants sprayed with poorly refined oils show that the rate of respiration was increased, but individual determinations show that in some cases respiration was retarded.

2. The averages of several tests with plants sprayed with highly refined oils also show an increase in the rate of respiration, but to a smaller extent than with poorly refined oils. Individual determinations, however, show that highly refined oils may also retard respiration.

3. The changes caused by oils in the rate of respiration extend over long periods.

4. When large quantities of oil were sprayed on barley seedlings about the same change in respiration was produced as when small quantities were used.

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CHANGES IN CARBOHYDRATE CONTENT OF WHEAT PLANTS DURING THE PROCESS OF HARDENING FOR DROUGHT RESISTANCE[1]

I. M. VASSILIEV AND M. G. VASSILIEV

Introduction

The process of hardening for drought resistance results in many changes in the vegetative organs of plants. PRINGSHEIM (7) has noted that pumpkin seedlings, grown under conditions of insufficient moisture, had an increased osmotic pressure in the cells and a higher resistance to drought than check plants grown in a moist soil and humid atmosphere. Since then a number of investigations have been conducted dealing directly or indirectly with the physiology of hardening. FREY (1), for example, has established that under conditions of sufficient soil moisture, plants which previously had suffered from lack of water supply were transpiring more intensely than normal plants grown in a moist soil. Studies by TUMANOV (9) have shown that sunflower plants, which had been hardened by repeated wilting, transpired 73 per cent. more moisture than non-hardened plants; while buckwheat, which had suffered from periodic drought, had an increased transpiration of 35 per cent. The same investigator states that plants which had been exposed to recurrent wilting exhibited greater intensity of assimilation after their return to a normal state. For buckwheat this increase was 50 per cent. over normal plants.

TUMANOV observed also that hardened plants had smaller cells and consequently a larger number per unit leaf area and a denser network of veins. The reaction between various parts of the plant during development was likewise changed under the effect of hardening. The leaf blade was more developed than the stem, and the root system was larger than the above-ground portion of the plant. VASSILIEV (11) has noted that the stomata were fully open when wheat was grown with a continuous optimum soil moisture of 40 per cent. of the total moisture capacity of the soil. The same wheat grown in soil saturated to 70 per cent. of its capacity suffered from insufficient water supply and the stomata of the leaves were almost closed when the moisture content in the soil was decreased to 40 per cent.

These investigations present morphological evidence about the hardening process. But such gross studies of the nature of hardening, as for example the xerophytic structure, the increased capacity of gas exchange (through stomata), and a more intensive transpiration and assimilation,

[1] Contribution from the Laboratory of Plant Physiology, Institute of Grain Farming, Saratov, U.S.S.R.

should be amplified by more detailed physiological studies of hardened plants.

The purpose of the present investigation was to determine the changes in carbohydrate content of plants during the periods of hardening and recovery. Through the aid of carbohydrates the osmotic pressure of the plant cell may be regulated markedly. Carbohydrates are important also as defense substances, protecting the protoplasm from coagulation when it is influenced by various harmful factors. Hence to characterize more definitely the hardening process for drought resistance, it seems desirable to consider first the carbohydrates.

This investigation represents at the same time a continuation of previously published studies (**11, 12**) on the relationship of carbohydrate metabolism in wheat to soil moisture and weather conditions.

Procedure

The experimental plants were various varieties of wheat differing in their resistance to drought. They were grown in pots containing 7 kilograms of soil, were fertilized (NPK) and watered daily to maintain approximately 70 per cent. of the moisture capacity of the soil. In each container were planted all the tested varieties. Reduction in soil moisture was obtained by discontinuing the water supply. The containers were weighed daily and the moisture content adjusted as desired. Samples were taken simultaneously from all the series under comparison. The plants were cut near the ground level and used promptly.

For the determination of moisture content the plants were weighed, dried at a temperature of 100° C., and reweighed. In the calculation of water content, the dry weight of 100 gm. was taken as the basis from which to compute departures. The material was prepared for analysis according to KISEL'S (**3**) method, as follows. The cut plants were put in an autoclave and killed by exposing to steam for 15 minutes. They were then hung up in a greenhouse, air-dried, and ground to fine powder which would pass through a sieve of 0.25 mm. mesh. The powder samples were preserved in desiccators until analyzed.

The analyses were conducted according to the procedure described by VASSILIEV (**11**), with the following changes: After separation from the insoluble fraction the filtrate was cleared, while passing through the filter, with animal charcoal, as suggested by LEHMANN (**5**). In addition to sugars, there were also determined the hemicelluloses by the following method: After removal of sugars, the residue was extracted with 2 per cent. of HCl on a water bath for three hours. When cool, the solution was filtered, neutralized with NaOH, and then filtered with suction a second time through animal charcoal. Further procedure with this extract was as

usual. In all cases the analyses were made on the total above-ground part of the plants. Samples up to June 1 were from plants in the stage of tillering. The carbohydrate data were calculated as milligrams per gram of dry tissue.

Results

The experiments deal with three stages of the process of hardening of plants: (1) the end of the period of loss of water; (2) the succeeding day after water was applied; and (3) eight days after irrigation, when the plants should have recovered from drought.

I. Changes in carbohydrate content of wheat during period of water loss

Experiment A (May 24).—On May 6, four days after the appearance of the seedlings above-ground, irrigation of a selected number of cultures was discontinued, so that the soil moisture in these containers gradually decreased. As the number of plants per pot was not large and the weather was cool, the loss of water proceeded slowly. On May 23, at noon, the plants had lost their turgor for the first time. The soil moisture had now decreased from 70 to 36 per cent. of the total moisture capacity of the soil. On the following day, at 2:30 P.M., samples were cut from these plants and from check plants growing under conditions of sufficient water supply (watered daily to 70 per cent. of soil capacity). The results of the determination of moisture and carbohydrate content are presented in table I.

Table I shows that owing to insufficient soil moisture the water content of all plants decreased about 30–40 per cent. The total carbohydrates of the experimental plants appear to have increased in the majority of cases. Only in one instance, variety Kitchener, the least resistant type, was there a reverse condition. The total soluble carbohydrates (sugars) increased in all experimental plants and in some cases this increase was very marked, as for example in the variety Sarroubra. The two groups of sugars, monosaccharides and sucrose, which made up the total soluble carbohydrates, were with few exceptions higher in the experimental than in the check plants. The relative quantity of hemicelluloses, on the contrary, decreased in the experimental plants.

The general conclusions from this experiment are as follows: With the gradual drying up of the soil in which this wheat was raised the water content of the plants had decreased approximately 35 per cent. at the time of wilting. The total sugar content had increased in all cases, the hemicelluloses decreased, and monosaccharides, sucrose, and total carbohydrates increased in the greatest number of varieties (one exception).

TABLE I

CHANGES IN MOISTURE CONTENT AND CARBOHYDRATES OF WHEAT PLANTS DURING PERIOD OF DROUGHT, MAY 24 (CARBOHYDRATES IN MILLIGRAMS PER GRAM OF DRY WEIGHT)

VARIETY	WATER		TOTAL CARBOHYDRATES	TOTAL SUGARS	HEMICELLULOSES	MONOSACCHARIDES	SUCROSE
	ABSOLUTE	PERCENTAGE					
		%	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Kitchener							
Check	667	100	118.8	29.9	78.9	9.9	20.0
Experimental	437	69	98.5	34.7	63.8	17.0	17.7
Gemtchoujina							
Check	675	100	97.8	22.6	75.3	12.8	9.8
Experimental	423	63	98.7	24.9	73.8	10.1	14.8
Caesium—0111							
Check	657	100	97.5	17.8	79.7	9.2	8.5
Experimental	418	62	108.0	29.0	79.0	12.5	16.5
Sarroubra							
Check	655	100	90.3	18.3	72.0	7.1	11.2
Experimental	437	65	107.9	51.4	56.5	15.3	36.1
Erithrospermum—341							
Check	669	100	85.1	14.8	70.3	7.1	7.7
Experimental	450	67	105.2	32.8	72.2	24.0	12.6

EXPERIMENT B (MAY 25).—This experiment was a continuation of the preceding one. The plants that began to wilt on May 23 were left without water for 24 hours. On May 25, at 2:30 P.M., or 24 hours later than the time of harvesting of the previous sample, another group of plants was cut for analysis. These specimens had a very wilted appearance and the tips of the leaves had begun to die. The results of analyses of this group are presented in table II.

It is evident from the figures in table II that the water content of the experimental plants has decreased still further and is now about 50 per cent. of the normal amount. The total carbohydrates, on the contrary, have increased, except in the variety Kitchener. But the total soluble carbohydrates (sugars) show an increase only in three varieties, two of the less drought resistant ones (Kitchener and Gemtchoujina) indicating a decrease, which was due to a conspicuous reduction in their sucrose content. And since the increase in sugar of the variety Sarroubra is slight,

TABLE II

CHANGES IN MOISTURE CONTENT AND CARBOHYDRATES OF WHEAT PLANTS AFFECTED BY EXTREME DROUGHT, MAY 25 (CARBOHYDRATES IN MILLIGRAMS PER GRAM OF DRY WEIGHT)

VARIETY	WATER		TOTAL CARBOHYDRATES	TOTAL SUGARS	HEMICELLULOSES	MONOSACCHARIDES	SUCROSE
	ABSOLUTE	PERCENTAGE					
		%	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Kitchener							
Check	681	100	144.7	49.1	95.3	18.4	30.7
Experimental	365	52	139.8	38.8	101.1	20.7	18.1
Gemtchoujina							
Check	636	100	125.4	39.9	85.5	17.3	22.7
Experimental	338	53	131.8	34.4	97.4	19.6	14.8
Caesium—0111							
Check	642	100	133.3	42.9	90.4	15.2	27.7
Experimental	347	52	173.9	67.9	106.0	22.3	45.6
Sarroubra							
Check	637	100	126.1	46.9	79.2	17.1	29.8
Experimental	367	58	58.5	47.9	110.6	22.9	25.0
Erithrospermum—341							
Check	678	100	119.0	30.6	88.5	11.6	18.9
Experimental	387	57	138.3	43.7	94.6	20.2	23.5

only two varieties showed a greater amount of sucrose than the check plants. Monosaccharides and hemicelluloses were present in larger quantities in all the experimental plants. Whereas the hemicellulose content in the experimental plants of the previous group was lower, it is now higher in most varieties.

The principal conclusions are: An increased loss of soil moisture has caused further decrease in the water content of the plants. This has resulted in continuous decrease in sucrose, which was noted in the first experiment, and an increase in monosaccharides and hemicelluloses. But the total carbohydrate content has decreased in most instances.

II. CHANGES IN CARBOHYDRATE CONTENT RESULTING FROM IRRIGATION OF WHEAT PLANTS THAT HAD SUFFERED FROM DROUGHT

EXPERIMENT C (MAY 24).—A group of plants which had suffered from drought were irrigated on May 23 to the same extent that normal check

plants were supplied with water (70 per cent. soil saturation). On May 24, at 2:30 P.M., samples of these plants were gathered for analysis. Table III gives the comparative records on moisture and carbohydrate content of the irrigated and non-irrigated (drought affected) plants.

TABLE III

CHANGES IN MOISTURE AND CARBOHYDRATE CONTENT OF WHEAT PLANTS AFFECTED BY DROUGHT AND SUBSEQUENTLY IRRIGATED (CARBOHYDRATES IN MILLIGRAMS PER GRAM OF DRY WEIGHT)

VARIETY	WATER		TOTAL CARBOHYDRATES	TOTAL SUGARS	HEMICELLULOSES	MONOSACCHARIDES	SUCROSE
	ABSOLUTE	PERCENTAGE					
		%	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Kitchener							
Wilting	437	100	98.5	34.7	63.8	17.0	7.7
Irrigated	584	133	126.4	38.3	88.1	7.0	31.2
Gemtchoujina							
Wilting	423	100	98.7	24.9	73.8	10.1	14.8
Irrigated	564	133	113.3	17.6	95.7	5.7	11.9
Caesium-0111							
Wilting	418	100	108.0	29.0	79.0	12.5	16.5
Irrigated	564	134	123.0	21.2	101.9	5.3	15.4
Sarroubra							
Wilting	437	100	107.9	51.4	56.5	15.3	36.1
Irrigated	560	128	.....	15.0	.....	5.2	12.8
Erithrospermum-341							
Wilting	450	100	105.2	32.8	72.2	24.0	12.6
Irrigated	581	129	146.8	23.6	105.4	5.2	9.8

The data of table III show that, as a result of irrigation, the moisture content, total carbohydrates, and hemicelluloses increased in all varieties while sucrose and total sugars decreased in practically every case and there was a decided reduction in the monosaccharides.

Comparison of the water and carbohydrate contents of plants which had received a deficient water supply, but had subsequently been irrigated, with that of normal (check) plants is given in table IV.

It is apparent from table IV that although the experimental plants had been irrigated on the previous day they were still deficient in moisture content, yet they indicate a remarkable augmentation in carbohydrates, all of them having increased in most groups excepting the monosaccharides.

TABLE IV

WATER AND CARBOHYDRATE CONTENTS OF WHEAT PLANTS GROWN WITH DEFICIENT (EXPERIMENTAL) AND AMPLE (CHECK) WATER SUPPLY

VARIETY	WATER		TOTAL CARBOHYDRATES	TOTAL SUGARS	HEMICELLULOSES	MONOSACCHARIDES	SUCROSE
	ABSOLUTE	PERCENTAGE					
		%	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Kitchener							
Check	667	100	118.8	29.9	78.9	9.9	20.4
Experimental	584	88	126.4	38.3	88.1	7.0	31.2
Gemtchoujina							
Check	675	100	97.8	22.6	75.2	12.8	9.8
Experimental	564	84	113.3	17.6	95.7	5.7	11.0
Caesium-0111							
Check	657	100	97.5	17.8	79.7	9.2	8.6
Experimental	465	86	123.1	21.2	101.9	5.8	15.4
Sarroubra							
Check	655	100	90.3	18.3	72.0	7.1	11.2
Experimental	560	86	.....	15.0	.....	5.2	12.8
Erithrospermum-341							
Check	669	100	85.1	14.8	70.3	7.1	7.7
Experimental	581	87	146.8	23.6	105.4	5.2	9.8

TABLE V

WATER AND CARBOHYDRATE CONTENT OF WHEAT PLANTS APPARENTLY RECOVERED FROM DROUGHT (CARBOHYDRATES IN MILLIGRAMS PER GRAM OF DRY WEIGHT)

VARIETY	WATER		TOTAL CARBOHYDRATES	TOTAL SUGARS	HEMICELLULOSES	MONOSACCHARIDES	SUCROSE
	ABSOLUTE	PERCENTAGE					
		%	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Kitchener							
Check	593	100	118.7	35.4	83.3	97.4	8.0
Experimental	545	92	135.8	38.9	96.9	22.7	16.2
Gemtchoujina							
Check	608	100	116.5	44.5	72.0	28.1	16.4
Experimental	537	88	164.6	61.1	103.0	20.6	40.5
Caesium-0111							
Check	591	100	117.8	47.6	70.4	28.4	19.2
Experimental	538	91	148.9	57.8	91.1	21.2	36.6
Sarroubra							
Check	593	100	176.2	52.2	124.0	26.0	26.2
Experimental	502	85	180.6	56.1	124.5	22.7	33.4

III. Water and carbohydrate content of wheat plants recovered from drought effects due to irrigation

Experiment D (June 1).—A group of plants which had been affected by drought were watered daily, beginning May 24, in the same way as the check cultures. On June 1, at 4:00 P.M., samples were gathered simultaneously from these and the check plants. The experimental plants were now of normal green color and were growing rapidly. The results of analyses are presented in table V.

These records (table V) permit the following conclusions: Plants which had been exposed to drought but later received an ample water supply, after eight days of irrigation were still deficient in moisture content. They were, however, much higher in most forms of carbohydrates, excepting the monosaccharides.

Discussion

The process of hardening plants for drought resistance may be divided into two phases: (1) the stage of water deficiency and (2) the stage of return to normal condition. During the period of water shortage, the plant is creating an internal state best suited for resistance to drought. The speed of this adjustment will depend on the rapidity of loss of water, but the trend is the same. The general direction of the changes in the vegetative organs is toward an increase of the suction tension of the cells and the water holding capacity of the tissues (Hauck **2**, and Lebedinzev **4**). This internal condition is maintained to a large extent even when the plants are restored to a normal supply of soil moisture. The results of the present studies will be considered on the basis of these statements.

The wheat plants exposed to drought in these experiments lost water slowly and began to wilt on the seventeenth day after irrigation was discontinued. On the following day the carbohydrate composition of these plants indicated an increased sugar (monosaccharide and sucrose) and a somewhat decreased hemicellulose content.

During the period of gradual loss of moisture the plants exhibited the following changes in their sugar content. First a decrease in both forms of sugars, which evidently was due to a decrease in photosynthesis. This change took place before the appearance of the first external signs of shortage of water, the wilting of the plants. The next step was the accumulation of sucrose and the consequent decrease in monosaccharides. The plants had now begun to wilt. This was followed by increase in monosaccharides and a simultaneous decrease in sucrose. Finally there was a gradual disappearance of the monosaccharides and the loss of sugar by the tissues. This stage began when the wheat suffered greatly from loss of water. It ended with the drying up and the death of certain parts of the plant. These results are in agreement with those previously reported (**11**).

The present investigation is of special interest in respect to the change in hemicellulose content. In our former work the hemicelluloses were not determined but a supposition was made that they were of the greatest importance in the carbohydrate balance of wheat plants. They are present in considerable quantity and seem to change in amount during the process of loss of water by the plant.

Results of the first experiment (experiment A) show a decreased quantity of hemicelluloses and an increased sugar content in most of the experimental plants as compared with the check plants. It is very probable that a correlation exists between the decrease in hemicelluloses and the increase in sugars in these plants, and that the hemicelluloses were at least one of the sources for the formation of sugars. Several investigators have shown that starch is present in the vegetative organs of wheat in but very small amounts and that it has no importance as a carbohydrate storage product. One may presume, therefore, that in wheat the hemicelluloses take the place of starch.

Results of experiment B present evidence of the conversion of sugars—the disappearance of sucrose. The hemicellulose content has also altered. But instead of a decrease, there is an increase in its quantity. This interesting fact may be interpreted in the following way: It is well known that hemicelluloses belong to a group of carbohydrate compounds the characteristic of which is a marked capacity to swell (the pentosans). ROSA (**8**) has assigned to them a decisive rôle in the increase in frost resistance of hardened plants. In a recent investigation by WOOD (**13**) on the carbohydrate balance of Australian succulent xerophytes, an unusually large accumulation of pentosans was noted in these plants when the aridity of the environment increased. The significance of the hydrophyllous colloids in the drought resistance of plants, especially wheat, is emphasized by NEWTON and MARTIN (**6**). From this and other studies we may conclude that the accumulation of hemicelluloses during the stage of water loss represents a means of resistance and a natural reaction of wheat plants to drought.

Let us now consider the carbohydrate balance of our plants during the period of recovery from drought. As soon as the plants were irrigated fundamental changes in the water and carbohydrate balance were brought about. The water content increased but the sugars, especially monosaccharides, decreased, while the hemicelluloses increased very markedly, thus producing an increase in total carbohydrates. These changes are not analogous to those existing in normal check plants. Compared with the latter, the experimental (irrigated) plants had a lower water and monosaccharides content but they were high in hemicelluloses, sucrose, and total carbohydrates. The same differences were still obtaining during the eighth day of recovery. Apparently the condition had become fixed in these plants.

The present data are inadequate to explain why the plants which had

been exposed to drought did not return after irrigation to the state of the check plants in their chemical composition. One may only suggest that the condition established during the drought period had become more or less irreversible.

In general, the most conspicuous feature by which a wheat plant that has been hardened to resist drought differs from a normal one is the greater accumulation of hemicelluloses and sugars, chiefly sucrose.

Summary

1. The object of this investigation was the determination of changes in carbohydrates of five varieties of wheat during the process of hardening for drought resistance.

2. Hardening was induced by growing the plants with insufficient water supply in suitable containers until permanent wilting had set in and the tips of the leaves had begun to die. Recovery was brought about by irrigation from this point on.

3. Representative numbers of plants were cut and analyzed for moisture content and carbohydrates at four periods: (1) the time of beginning of wilting, (2) the stage of permanent wilting, (3) twenty-four hours after resumption of irrigation, and (4) eight days after recovery. The carbohydrate analyses consisted of quantitative determination of monosaccharides, sucrose, and hemicelluloses, the most important constituents of the wheat plant.

4. On the day following the beginning of wilting the carbohydrate content of the plants of most varieties studied was as follows: An increased concentration of both forms of sugar (monosaccharides and sucrose) and a somewhat decreased concentration of hemicelluloses. With further loss of water, when the points of the leaves commenced to yellow, there were the following changes: Decrease in sucrose, increase in monosaccharides, and a decided increase in hemicelluloses; the contents of the latter were now greater than in the check plants. On the day following the first irrigation, the water content in the tissues had increased but not to the amount present in the check plants. The monosaccharides had decreased and sucrose was also at lower concentration, and in most cases less than in the control plants. After recovery (eight days of irrigation following maximal drought effects), the experimental plants, when compared with normal plants, still had a decreased water and monosaccharide content and an increased concentration of sucrose and hemicelluloses. This condition had become more or less "fixed."

5. The significance of the hemicelluloses in drought resistance of wheat is discussed.

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EFFECT OF VARIATION IN LENGTH OF DAY ON GROWTH AND DORMANCY OF TREES

PAUL J. KRAMER

(WITH FOUR FIGURES)

Introduction

One of the outstanding characteristics of the woody plants that are native to the temperate zone is the periodicity of their growth. This periodicity has commonly been assumed to depend largely if not entirely on the periodicity of temperatures favorable to growth. There are reasons, however, for doubting that temperature is the only important factor affecting the length of the growing season. Even in the tropics where the temperature is always high enough to permit growth, many species show a definite periodicity. In the temperate zone the growing season of most species is relatively short, growth ceasing and in some cases leaf abscission beginning long before the occurrence of frosts. Even evergreen species have a limited growing season which usually ends before the advent of temperatures low enough to check growth appreciably. Furthermore, many species of trees brought into the greenhouse in the summer and kept there during the autumn and winter cease stem elongation and the production of new leaves as soon as trees of the same species that are left out-of-doors.

Dormancy in such cases is evidently not the result of decreasing temperature, but is caused by some other factor which operates inside as well as outside the greenhouse. The only such factor which varies with the season is length of day. In the latitude of Durham, North Carolina, where these observations were made, there is a difference of about five hours between the longest and the shortest days of the year and this difference becomes progressively greater with increasing distance from the equator.

As a result of the work of GARNER and ALLARD (**6, 7**) and others, it is well known that the length of day or photoperiod is an important factor in determining the time of flowering and amount and nature of vegetative growth of many species of herbaceous plants. References to the extensive literature on this subject are given in the papers of GARNER and ALLARD (**6, 7**), KELLERMAN (**11**), LAURIE and POESCH (**16**), and RAMALEY (**21**), so no review of the work on herbaceous plants will be made here.

KLEBS (**13**) was one of the first to investigate the effects of length of day on woody species. He reported that beech, oak, ash, and hornbeam grew all winter when placed under continuous electric light. GARNER and ALLARD (**7**) also experimented with several species of woody plants, including apple, yellow poplar, box elder, winged sumac, and smooth sumac. They found that yellow poplar, given daylight supplemented by electric light

until midnight, grew all winter, while smooth sumac treated in the same manner retained its leaves during the winter but made no growth. Leaf fall was retarded only a few weeks by this treatment in the case of winged sumac. Apple made more growth with a 10-hour day than with the normal long days of midsummer, but box elder grew better with the long summer days. GARNER and ALLARD concluded that the length of day might be an important factor in determining the time both of beginning and of ending the dormant period. ADAMS (**1**) reported that sugar maple given electric light at night produced leaves no sooner in the spring than trees receiving only daylight. MOSHKOV (**17, 18, 19**) reported that certain southern tree species, such as *Robinia pseudoacacia* and *Salix babylonica,* when grown at Leningrad where the maximum length of day is 20 hours, continued to grow until killed by frost instead of becoming dormant at the end of the summer. If the photoperiod was artificially shortened such species ceased growth earlier in the season and were not injured by cold. He also found that the morphology of certain species was altered. *Robinia,* for instance, with short days branched less than with normal day, formed scales instead of thorns, and produced fewer pairs of leaflets. BOGDANOV (**3**) also found that the length of growing season and resistance to cold of a number of species of trees were affected by the photoperiod with which the plants were grown. Further details of the Russian work may be found in a recent paper by GEVORKIANTZ and ROE (**9**). DARROW and WALDO (**4**) found reason to believe that the rest period in the strawberry may be a result of nutritional conditions caused by short days and low temperatures. They believed that this might also be true of the rest period of other plants, including woody ones.

Since so few species had been investigated, it seemed worth while to investigate the behavior of other species under various lengths of day. The species studied were: white ash (*Fraxinus americana* L.), green ash (*F. pennsylvanica* var. *lanceolata* Sarg.), beech (*Fagus grandifolia* Ehrb.), black locust (*Robinia pseudoacacia* L.), yellow poplar (*Liriodendron tulipifera* L.), red or sweet gum (*Liquidambar styraciflua* L.), post oak (*Quercus stellata* Wang.), northern red oak (*Q. borealis maxima* Ashe), white oak (*Q. alba* L.), and loblolly pine (*Pinus taeda* L.).

The experiments with these species extended over three growing seasons, although not all of the species were studied through all three seasons. The following report is a brief summary of the important results obtained during the three seasons.

Methods and results

Most of the plants used in these experiments were one or two years old, but a few of the oaks and red gums were grown from seed. All the plants

were grown in ordinary garden soil in 6- and 8-inch clay pots, one plant being grown in each pot.

The most extensive series of experiments was conducted in the following manner. During the early part of the summer, groups of 10 to 15 trees of each species to be studied were placed under various lengths of day. One series received the normal length of day, which varies from 14½ hours in June to 9½ hours in December. Another series received full daylight supplemented by electric light. The electric light was controlled by an automatic time switch and as the days changed in length the lights were turned on for a longer or shorter period of time, so that the plants continued to receive approximately 14½ hours of light or a photoperiod equal to the longest summer days. The light was supplied by ordinary 200-watt mazda lamps. The light intensity at the surface of the soil varied from 100 to 200 foot-candles. A third series was given an 8½-hour day by covering the trees from 4:45 P. M. to 8:15 A. M. with black cloth shades suspended over a framework about 6 feet above the plants. All the plants were subjected to the daily and seasonal variations in temperature which usually occur in a greenhouse. The differences in temperature between the groups under different lengths of day did not exceed two or three degrees. Appreciable variations in temperature are undesirable, for BERKELEY (**2**), GILBERT (**10**), and others have shown that differences in temperature may exert a marked effect on photoperiodic reaction.

All of the species just listed were grown under normal length of day. Stem elongation and the production of new leaves ceased in the autumn as soon in those trees growing in a warm greenhouse as in trees of the same species left out-of-doors. The leaves of beech and yellow poplar abscissed as soon indoors as outside, but the two species of ash, yellow locust, red oak and post oak retained their leaves in a living condition somewhat longer indoors than outside. Most of the leaves of the red gum and white oak remained green until early spring when new leaves began to appear.

White ash, yellow poplar, red gum, post oak, white oak, and loblolly pine were grown under short day conditions. All of these species except white oak made less growth and became dormant sooner than the trees exposed to normal length of day. There was no appreciable difference between the normal and short day white oak.

All of the species received long day treatment and all of those so treated except white and green ash and red oak grew later in the autumn and made greater shoot growth than trees exposed to the normal photoperiod. Loblolly pine grew all winter with the 14½-hour day (figs. 1, 2). Yellow locust and yellow poplar continued to grow until late December when an outbreak of mealy bugs and red spiders killed most of the leaves. Other experiments indicate that with a 16-hour day these species will grow all winter.

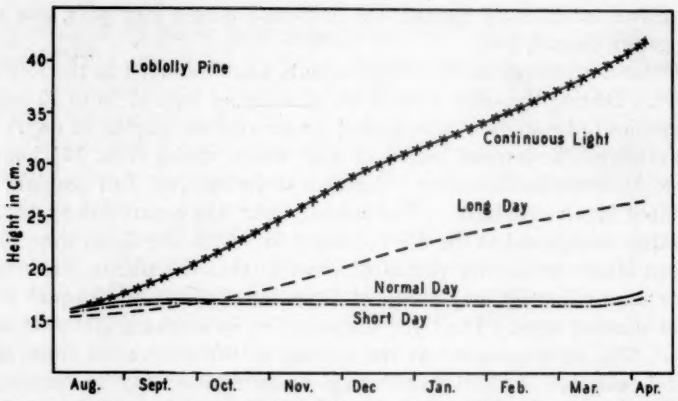

FIG. 1. Shoot growth of loblolly pine with photoperiods of various lengths.

In another series of experiments red gum, yellow poplar, and post oak were exposed to electric light until midnight. The yellow poplar continued stem elongation and the production of new leaves during the entire winter and the red gum and post oak continued to grow much later in the autumn than the trees receiving the normal length of day. Yellow poplar, loblolly pine, and red gum given electric light all night made even more growth than those receiving long day (figs. 1–3). The long day post oak did slightly better than those receiving continuous light, but this experiment must be repeated to determine whether or not this is the characteristic reaction. The growing season of green ash was not lengthened by continuous light. There was a tendency for some of the red gum plants to cease growth toward spring and become dormant for a time. This might possibly have resulted from a deficiency of minerals, but further investigation of this is necessary.

Two series containing yellow poplar, red gum, red oak, and white ash were placed outside the greenhouse. One series received the normal length of day, while the other series received a 14½-hour day. The red gum and yellow poplar grew about two weeks later in the autumn with extra light, but the growing season of the red oak and white ash was not extended. In the spring there was no difference between the time at which dormancy was broken in the normal and in the long day series. Apparently the days had already lengthened sufficiently no longer to constitute a limiting factor, and all of the trees were in a condition to resume growth as soon as the temperature was favorable.

Dormant seedlings of beech, yellow poplar, red gum, and red oak were brought indoors early in January and subjected to long and short days. In all instances the trees receiving additional light resumed growth sooner

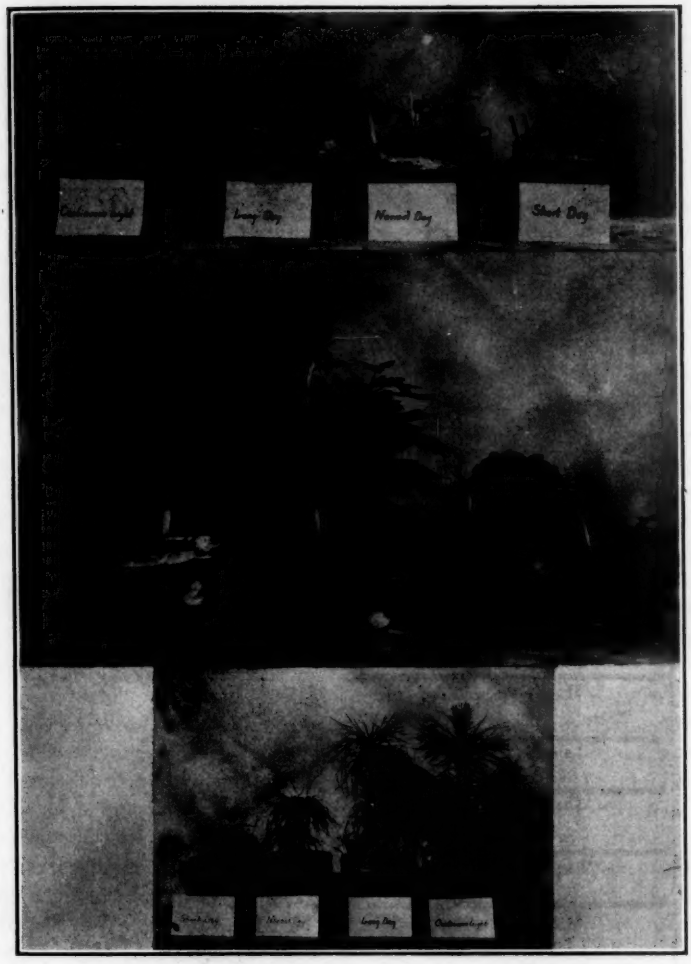

FIG. 2. *A*, post oak; *B*, red gum. In *A* and *B* the photoperiods are, from left to right: continuous light, long day, normal day, short day. *C*, loblolly pine. From left to right the photoperiods are: short day, normal day, long day, continuous light.

than those with a short day. Some of the results of this experiment are shown in figure 4. Certain short day red gum trees kept in the greenhouse all winter resumed growth as soon as normal day trees, but only the lateral buds opened, growth was very slow, and the leaves were small in size

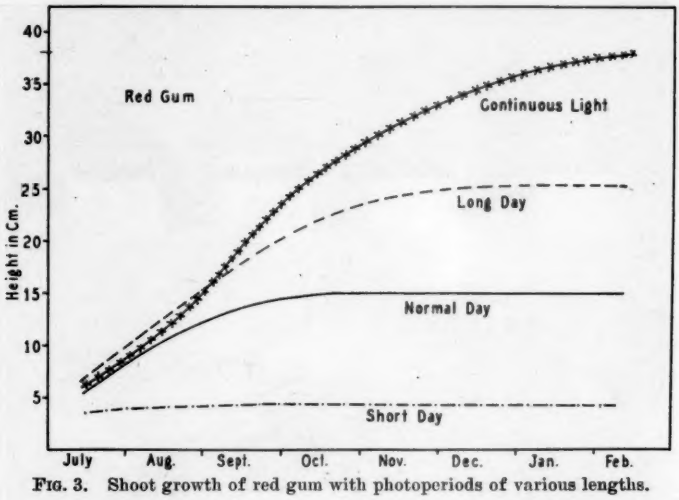

FIG. 3. Shoot growth of red gum with photoperiods of various lengths.

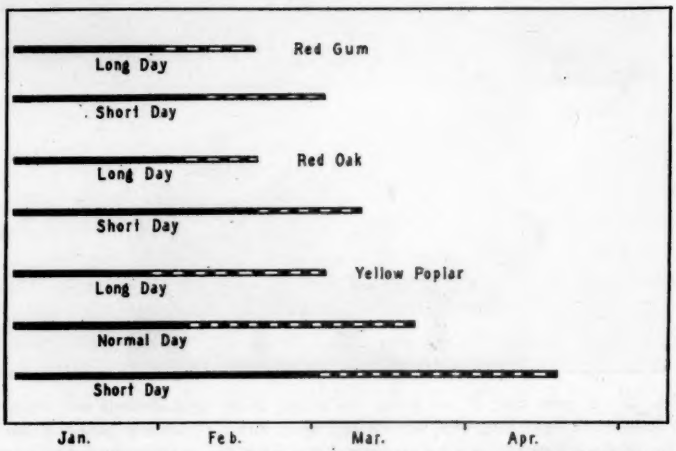

FIG. 4. Length of time required to break dormancy in trees brought into the greenhouse and subjected to various photoperiods. Solid line represents completely dormant condition; barred line, interval from opening of first buds until all buds were open.

and pale in color. These trees never caught up with the trees receiving normal or long photoperiods.

Discussion

The results of these experiments indicate that with certain species the time at which dormancy begins and ends is closely related to the length of day. Certain other species such as white and green ash do not seem to be affected by variations in length of photoperiod. It is possible either that these species are not sensitive to length of day or that the proper combination of photoperiod and temperature has not been tried. A classification of the species studied into long and short day types is not possible at present. Thus far the reaction to an increased photoperiod has been much more marked than has the reaction to a shortened photoperiod. GARNER and ALLARD (**7**), however, found that a certain variety of apple made more growth with a 10-hour day than with a 14- or 15-hour day, and other cases of this type will doubtless be found. As pointed out by GARNER (**5**), the original classification of GARNER and ALLARD is based on the effect of length of day in initiating or suppressing sexual reproduction. The studies on trees have thus far been concerned solely with length of growing season and amount of vegetative growth, and it may be questioned whether or not they should be classified as long or short day types on this basis. Since we are chiefly interested in their vegetative growth, however, and since there are obvious difficulties which will probably prevent any very extensive investigation of the response of flowering and fruiting to length of day, it may be justifiable to classify them as long, short, or insensitive types on the basis of vegetative growth.

It is probable that in nature the beginning of dormancy is more dependent on length of day than is the ending of dormancy. The shortening days of late summer may bring about the cessation of growth in some of the more sensitive species considerably before it would be stopped by decreasing temperature alone. On the other hand, in many instances it is probably the occurrence of temperatures high enough for growth rather than the length of day which determines the time of resumption of growth in the spring. It is likely that in some regions the days have lengthened sufficiently no longer to constitute a limiting factor before the temperature is high enough to permit the full resumption of growth.

It seems possible that differences in the length of the growing season of various species in the same locality may result from differences in their reaction to seasonal variations in photoperiod. KIENHOLZ (**12**), working at Keene, New Hampshire, found that *Pinus resinosa* began growth in late April and ceased growth about the middle of August, with a total growing season of 105 days; while *Abies balsamea* began growth late in May and ceased growth late in July, with a growing season of 65 days. These differences could scarcely have resulted from differences in temperature, moisture, or

the supply of minerals, but may have been caused by differences in tolerance to length of day.

Although length of day seems definitely to control the length of growing season of some tree species, it should be regarded as an *indirect* rather than as a *direct* causal agency in initiating or breaking dormancy. Changes in dormancy, flowering and fruiting, root-shoot ratios, and other growth phenomena are the direct result of changes in the internal physico-chemical processes and conditions of the protoplasm. Within the limits of the hereditary potentialities of any particular species or individual, such changes in internal processes and conditions may be brought about by one or several environmental factors acting singly or in combination. This viewpoint was set forth by KLEBS (**14**) many years ago. It is supported by the work of ROBERTS (**22**), who found that no separate environment had a consistently unique effect upon the internal composition or behavior of apple trees, but that similar changes in composition and behavior might result from different treatments. Thus short day trees not supplied with nitrogen behaved much like long day trees with nitrogen.

It may be that a state of equilibrium exists in respect to the complex of internal physiological processes, and by shifting this equilibrium in one direction dormancy results while a shift in the other direction results in the resumption of growth. Several different environmental factors acting separately or in combination may produce a sufficient shift in this equilibrium to result in cessation or resumption of growth. The most important factors are probably the supply of water and minerals (especially nitrogen), the temperature, and the photoperiod.

As yet the manner in which variation in length of day produces such marked reactions in plant behavior has not been satisfactorily explained. Experiments on herbaceous plants have shown that there are changes in hydrogen-ion concentration and hydration of tissues (**8**), carbohydrate-nitrogen relations (**20, 24**), root-shoot ratios (**25**), and catalase activity (**15**), and similar changes doubtless occur in woody plants. The rate and amount of photosynthesis is doubtless affected and it has been shown that in some plants the utilization of nitrates is also affected (**20**). ROBERTS and KRAUS (**23**) have suggested that photoperiodic responses are caused by changes in type of daily respiratory rhythms. These changes in rate of respiration are probably not the cause but the result of more fundamental changes resulting from photoperiodic activity. It is probable, however, that all these phenomena are correlations or indirect causes and that the real cause lies in changes in cell metabolism. Much more work on the fundamental physiological processes governing growth will be necessary before this problem can be explained.

It seems that information concerning the photoperiodic reactions of

various tree species would be of use in several ways. It may explain differences in length of growing season of various species growing in the same region. It may also explain why some species do not thrive when moved to a different latitude even though climatic conditions seem favorable. Knowledge of the optimum photoperiod should aid in predicting whether or not a given species is suitable for growth at any given latitude. In connection with research in forestry, horticulture, and pathology, it is frequently desirable to control the growing season so that growing material is available whenever needed. This can be done with some species by exposing them to the proper photoperiod. Seedlings of some species will also make much more rapid growth if given additional light and thus are ready for use sooner than if grown with normal length of day.

Summary

1. The behavior of potted seedlings of white and green ash, beech, yellow locust, yellow poplar, red gum, post oak, northern red oak, white oak, and loblolly pine was observed under various lengths of day. With normal length of day all of these species ceased growth as early in the autumn in a warm greenhouse as out-of-doors, although most species did not lose their leaves as soon indoors. All of the species except white and green ash and red oak made more growth and grew later in the autumn when daylight was supplemented by electric light to give a total photoperiod of 14½ hours. Several of the species were grown with an 8½-hour day and most of these made less growth and became dormant sooner than with normal day.

2. With a 16-hour day, red gum grew nearly all winter and yellow poplar grew all winter; both species grew all winter with continuous light. Loblolly pine grew all winter with a 14½-hour day, but made better growth with continuous light.

3. Dormant trees of beech, yellow poplar, red gum, and red oak were placed under various lengths of day in midwinter. In all species resumption of growth was hastened by additional light and retarded by shortening the photoperiod below the normal length of day.

4. The results of these experiments indicate that variations in length of day may, to a considerable extent, control the duration of the growing season of certain tree species. These results also strongly support the view that dormancy is not caused by any inherent rhythm in plants, but is produced by the action of various environmental factors on the complex of internal physico-chemical phenomena controlling growth.

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DISTRIBUTION OF CERTAIN SUGARS IN BOSC PEARS

WILLIAM E. MARTIN

(WITH TWO FIGURES)

Introduction

Since an investigation of the various sugars present in winter pears was in progress at this station, it was thought desirable to determine in a preliminary way the variation in the sugar content of the various portions of the fruit, and from this information to decide as to the best method of taking samples of the pear. The effort was made to divide the fruit into natural regions on the basis of anatomical characteristics rather than on the basis of some arbitrary geometric differentiation.

KELHOFER (**6**) has noted that in the Siebenmannsbienen pear sugar and acid were higher in the central fleshy portion of the fruit than in the core or peel. Tannin was observed in greatest concentration in the peel while small amounts were noted in the core region. Analyses of blossom end, central portion, and stem end showed slightly more sugar and acid in the blossom end than in the central portion, and still smaller amounts in the stem end of the fruit.

ALLEN (**1**) in reporting on the electrical conductivity of pear tissue showed that there were quite marked differences in the specific resistance of the flesh of the cortical and pith regions of Bartlett pears, and noted that the resistance of the flesh of these regions changed as the fruit ripened after harvest.

While a number of workers have investigated the amounts of sucrose, dextrose, and levulose in pears both during the growing season (**5, 14**) and during storage (**4**), none have reported the concentration of these sugars in the several regions of the fruit.

SMITH (**13**) has recently published a comprehensive report dealing with the course of stone cell formation in pear fruits and has shown the changes in total sugars, starch, hemicellulose, pectins, and other constituents during growth and subsequent storage. He has pointed out the importance of expressing the results of analyses of growing fruits in terms of the actual amounts of the various constituents per fruit rather than in terms of concentration, and has suggested that sampling which included the whole fruit would be more representative in sugar content than that taken only from the midsection if it were established that marked differences in sugar concentration existed in the various parts of the fruit.

CRIST and BATJER (**3**) have reported on the nature and occurrence of "grit cells" in pear fruits and have discussed their probable function as well as that of the stony sheath of these cells surrounding the core of the fruit.

Since no complete investigation of the morphology of the pear has been reported as yet it was thought desirable to make a brief study of longitudinal and transverse sections of the pear in an effort to determine the general anatomy of the fruit. Longitudinal sections were prepared by cutting 4-mm. sections of the fruit with a bread knife, then soaking these overnight in 5 per cent. chloral hydrate, and finally clearing for one week in 2 per cent. potassium hydroxide. Transverse sections were made by imbedding a pear in a block of paraffin and sectioning in a meat slicer in one of the local butcher shops. By use of this method transverse sections 1 mm. thick from the calyx to the stem end of the fruit were rapidly and easily cut. These sections were partially dehydrated without curling by keeping in 70 per cent. alcohol 24 hours and then clearing in 95 per cent. alcohol for one week. By these methods both longitudinal and transverse sections were prepared which photographed clearly. The nomenclature used by KRAUS (**7, 8**) in his studies of the morphology of the apple has been adopted to designate and describe the various regions of the pear.

After a study of the longitudinal and transverse sections of the pear (figs. 1 and 2) it became evident that the fruit might logically be divided into four regions on the basis of marked differences in the anatomy of the fruit itself. The most obvious of these regions were the skin and core portions of the fruit, but it was also noticed that the remaining flesh was made up of two distinct areas differing markedly in their structure. The outer portion of

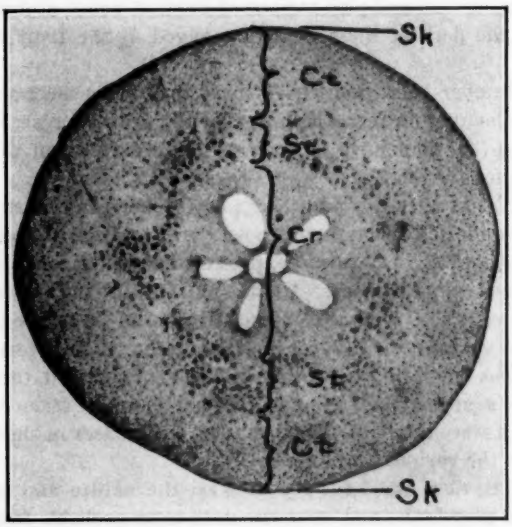

FIG. 1. Transverse section of pear through carpels.

the flesh contained a network of fine anastomosing vascular bundles and comparatively few small stone cells, while the inner portion was practically devoid of fine anastomosing bundles and contained many large stone cells which formed a rather dense sheath about the core. These two portions of the fruit were separated at the median section through the carpels (fig. 1) by the ten primary vascular bundles, but no definite core line was observed as in the apple.

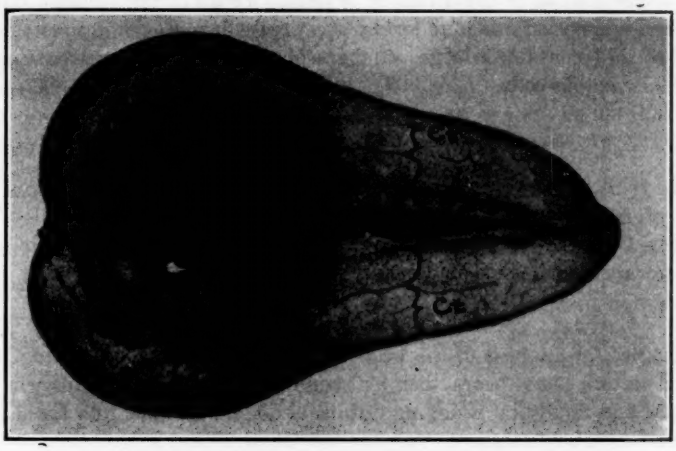

FIG. 2. Longitudinal section of pear along axis and through carpels.
Sk—Skin region
Ct—Cortical region
St—Stone cell region
Cr—Core region

Using these observations as a basis the fruit was divided into the following four regions: (a) The innermost portion of the fruit was designated as the *core region* and was made up of the seeds, carpels, and the surrounding parenchyma which extended outward to the dense stony layer mentioned above and shown in figures 1 and 2. (b) The outermost portion of the fruit was designated as the *skin region* and included the cuticle, epidermis, and such portions of the cortex as might be removed in careful peeling. The cuticle and epidermis had been ruptured in much of the skin during the natural process of russeting and a number of layers of corky tissue had been formed from a cork cambium in the outer portion of the cortex. These corky tissues were likewise included in the skin region. (c) The outer portion of the remaining flesh containing the secondary vascular bundles and their fine anastomosing branches extended inward as far as the outer cycle of primary vascular bundles and appeared to correspond to the cortex of the apple. It

was therefore designated as the *cortical region.* (d) The remaining portion of the fruit was designated as the *stone cell region.* In the calyx end of the fruit and at the section through the carpels (fig. 1) this region lay inside of the ten primary vascular bundles, but toward the stem end it was apparently traversed by the dorsal and ventral carpellary bundles as well as by the inner cycle of primary vascular bundles. From the material at hand it was not possible to determine whether the tissue of this region belonged morphologically to the pith or to the cortex or to both. In all of the sections studied, however, this region was found to be practically devoid of fine anastomosing bundles, and contained large numbers of stone cells or aggregations of sclerenchymatous cells.

Materials and methods

Source of materials

The fruit used in this investigation was obtained from a single tree of a block of full-bearing Bosc trees in the upper Rogue River Valley near Phoenix, Oregon. The fruit was picked on August 26, 1934, and withdrawn from storage for analysis on January 20, 1935.

Methods of sampling

Samples for analysis of the whole fruit were prepared by cutting longitudinal sectors of ten pears, then by grinding these through a Russwin food chopper, and finally by weighing duplicate 50-gm. samples into tared beakers on a laboratory balance.

In the preparation of samples of tissue from the four regions which have been described above, ten additional sectors were cut from the opposite sides of the same ten pears. These ten sectors were then divided by means of a small narrow-bladed scalpel into portions from the skin, cortical, stone cell, and core regions. The portions from each region were placed in tared beakers and the fresh weight of tissue from each region was recorded. Seven additional pears were sampled in the same fashion except that two opposite sectors were cut from each fruit. Enough additional skin was pared and core material cut from the remaining portions of the fruit so that sufficient material was at hand to prepare duplicate 25-gm. samples of the core material and duplicate 50-gm. samples of tissue from each of the other regions. All samples were preserved by boiling 2 to 3 min. in sufficient ethyl alcohol so that the final concentration was above 80 per cent.

Methods of chemical analysis

The material was extracted with 95 per cent. ethyl alcohol in a Soxhlet apparatus, the alcohol then evaporated at 40 to 45° C. under a 26 to 28-in. vacuum, and the aqueous extract finally clarified by the addition of neutral lead acetate and deleaded with a slight excess of potassium oxalate.

Reducing sugars before and after inversion were determined by the method of LANE and EYNON (9). Sucrose was then calculated from the difference in reducing sugars before and after inversion. The inversion was carried out by the following procedure: 10 ml. HCl (sp. gr. 1.1029) were added to 100 ml. of solution and the mixture heated one hour in a hot water oven at 70° C. Dextrose and levulose were determined by the method of LOTHROP and HOLMES (11) with the following modifications. (a) Owing to the low sugar content of the extracts to be analyzed it was found desirable to reduce by half the amounts of the reagents to react with a given amount of sugar solution. (b) Owing to lack of facilities it was necessary to carry out the oxidation at room temperature (20–25° C.) instead of at 20° C. Total sugars were computed as the numerical sum of sucrose, dextrose, and levulose. The alcohol insoluble residue was determined as the dry weight of the material remaining in the tared thimbles after extraction.

Presentation and discussion of results

The amounts and proportions of the fresh tissue found to be present in the various regions, as determined by the weights of the component parts of the longitudinal sectors, are listed in table I. It is to be noted that the cortical region contained over half of the total tissue of the pear, the stone cell region a little over a quarter, while successively smaller amounts were found in the skin and core regions.

TABLE I

REGIONAL DISTRIBUTION OF PEAR TISSUE

REGION	10 SECTORS FROM 10 PEARS		14 SECTORS FROM 7 PEARS		AVERAGE PERCENTAGE OF FRESH WEIGHT
	WEIGHT OF TISSUE	PERCENTAGE OF FRESH WEIGHT	WEIGHT OF TISSUE	PERCENTAGE OF FRESH WEIGHT	
	gm.	%	*gm.*	%	%
Skin	23	11.92	24	10.39	11.08
Cortical	104	53.88	126	54.55	54.25
Stone cell	53	27.46	64	27.70	27.60
Core	13	6.74	17	7.36	7.07
Total weight	193	100.00	231	100.00	100.00

The results of the sugar analyses of the material from each of the four regions of the fruit as well as those of the undivided sectors are shown in table II.

It is apparent from table II that the total sugar content was greatest in the cortical and core regions, while it was somewhat less in the stone cell region and markedly smaller in the skin.

TABLE II

VARIATION IN SUGAR CONTENT OF THE SEVERAL REGIONS OF THE PEAR EXPRESSED AS PERCENTAGES OF FRESH WEIGHT

REGION	ALC. INS. RESIDUE	SUCROSE	LEVULOSE	DEXTROSE	REDUCING SUGARS	TOTAL SUGARS
	%	%	%	%	%	%
Skin	5.82	1.91	3.27	3.72	6.99	8.90
Cortical	2.80	2.72	6.00	2.32	8.32	11.04
Stone cell	4.85	3.60	4.55	2.00	6.55	10.15
Core	5.52	2.25	5.80	2.67	8.47	10.72
Undivided sectors	3.98	3.18	5.22	2.39	7.61	10.79

More significant variations, however, were observed in the concentrations of the individual sugars. Sucrose was present in far greater amounts in the stone cell region than in any other part of the fruit. Both levulose and reducing sugars were present in largest amounts in the core and cortical regions.

The fact that both levulose and reducing sugars were high in the core and cortical regions is of especial interest since these portions of the fruit appear to be the terminal regions of the vascular bundles through which carbohydrates pass into the fruit. Similarly the facts that sucrose was high and that reducing sugars were low in the stone cell region are significant since this region appeared to be very nearly devoid of fine anastomosing bundles although it was traversed and bordered by the larger vascular bundles.

While dextrose was observed to be present in the highest concentration in the skin there is a possibility that some interfering substance may have been present. The glucoside arbutin, reported in pear bark by LINCOLN (**10**) and in pear leaves and in the skin of the pear by BOURQUELOT and FICHTENHOLZ (**2**), may have affected the observed proportions of levulose and dextrose if present in appreciable amounts. Tests with pure arbutin, added to pear extracts, showed that its presence would not have affected the values of reducing sugars or sucrose which were determined by copper reduction methods, but would have materially affected the iodometric determination of dextrose as carried out by the method of LOTHROP and HOLMES (**11**), and consequently would have caused error in the observed proportions of dextrose and levulose. Unfortunately no work was done in this preliminary investigation to determine the actual extent to which arbutin or other interfering substances were present in the fruit.

The composition of the undivided sectors, as shown in table II, differed considerably from that of tissue from the other regions studied. This was to be expected, however, since the undivided sectors were made up of unequal

amounts of material from each of these four regions of the fruit. It may be well to point out, in addition, that the sectors dissected to provide regional material were in no cases taken adjacent to the undivided sectors, and in over half of the material were taken from a different set of pears.

A comparison of the composition of the undivided sectors with the corresponding compositions of the tissues of the four regions was made in order to test the method of using longitudinal sectors as a source of representative material in sampling. These sectors taken along the longitudinal axis, should have contained material from the various regions of the fruit in proportion to the total amounts of material present in each region in the whole fruit. The composition of the undivided sectors should have been a weighted average of the individual compositions of the four designated regions.

To test this it was assumed from the data in table I that in 100 gm. of representative material, 11.08 gm. was derived from the skin region, 54.25 gm. from the cortical region, 27.58 gm. from the stone cell region, and 7.07 gm. from the core region. By multiplying each of these weights by the percentages of the several sugars listed in table II for the appropriate region, data were obtained showing the amount and source of the sugars observed in 100 gm. of representative tissue. These amounts are listed in table III.

TABLE III

DISTRIBUTION OF SUGARS IN 100 GRAMS OF REPRESENTATIVE TISSUE EXPRESSED IN GRAMS OF SUGAR

REGION	ALC. INS. RESIDUE	SUCROSE	LEVULOSE	DEXTROSE	REDUCING SUGARS	TOTAL SUGARS
Skin	0.643	0.211	0.361	0.411	0.772	0.983
Cortical	1.519	1.476	3.255	1.258	4.513	5.989
Stone cell	1.338	0.994	1.256	0.552	1.808	2.802
Core	0.390	0.159	0.410	0.189	0.599	0.758
Sum of the sugars in all regions	3.89	2.84	5.282	2.410	7.692	10.532
Composition of undivided sectors	3.98	3.18	5.22	2.39	7.61	10.79

Ideally the sum of the amounts of each sugar found in the four regions should have equalled the amount of the same sugar as determined by the analysis of the undivided longitudinal sectors. A comparison of these two sets of values is given in table III and it may be noted that these values are in most cases nearly equal. It appears then that the analysis of longitudinal sectors yields results very nearly equal to the sum of the same constituents as determined by the analysis of the component regions of the fruit.

If further investigation bears out the observed differences in sugar con-

tent noted in the several regions of the fruit, it would seem essential in taking samples for chemical analysis that each region make up the same proportion of the sample as of the total tissue of the fruit. Similarly it may be pointed out that the removal of the skin and core in sampling may affect the results of subsequent analyses, while the use of flesh of the fruit derived only from the midsection may yield samples which are not truly representative of the fruit as a whole.

Summary

1. As a result of a study of sections of the fruit, the pear was divided for chemical analysis into the following four regions on the basis of anatomical differences in structure.

(a) The *skin region* included the cuticle, epidermis, and several layers of corky tissues together with such additional portions of the cortex as might be removed in careful peeling.

(b) The *cortical region* included the bulk of the cortex and was located between the skin region and the outer cycle of primary vascular bundles and contained large numbers of fine anastomosing vascular bundles.

(c) The *stone cell region* included the tissues lying between the cortical region and the pith parenchyma about the carpels, and was so named because of the large numbers of stone cells occurring in that portion of the fruit.

(d) The *core region* included the carpels, seeds, and the pith parenchyma extending from the carpels outward to the dense stony sheath marking the inner boundary of the stone cell region.

2. The observed amounts of material found in these regions constituted far different percentages of the pear, 54 per cent. of the fresh weight being found in the cortical region, 28 per cent. in the stone cell region, 11 per cent. in the skin region, and 7 per cent. in the core region.

3. While noticeable differences in the total sugar concentration were observed in the four regions, far more significant differences were noted in the concentrations of the individual sugars, dextrose, levulose, and sucrose, in these regions.

4. Levulose was found to be the predominant sugar in all parts of the fruit except the skin, and the highest concentrations were noted in the cortical and core regions.

5. Sucrose was observed in largest amounts in the stone cell region with lessening amounts in the cortical, core, and skin regions.

6. Dextrose was present in largest amounts in the skin while the amounts found in the other regions differed but slightly.

7. On the basis of these preliminary observations, it appears highly desirable in taking samples of Bosc pears for chemical analysis that the amounts of material from each of the four regions of the fruit be present in proportion

to the total amounts of tissue of each of these regions actually present in the fruit if a truly representative sample is to be obtained.

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INFLUENCE OF THE CHLORIDE ION ON THE CONTENT OF CHLOROPHYLL IN THE LEAVES OF POTATOES[1]

S. Basslavskaya and M. Syroeshkina

(WITH ONE FIGURE)

Introduction

The color of plants depends to a great extent on the conditions of nutrition. Approximately fifty years ago Ville (7) attempted to determine the proper provision of plants with elements of nutrition by their color from the amount of chlorophyll produced.

The pale color of plants may depend on various causes. In some cases it is called forth by an actual reduction of the amount of chlorophyll owing to the depressive action of one or another factor on the formation of chlorophyll. However, very often the pale color is conditioned not by the decrease of the amount of chlorophyll, but by other factors, such as the accumulation of colored substances, increase of water content, and so on. The ability to determine these factors is of great importance when studying the changes in color called forth by potassium salts, especially by chlorides, which greatly increase the water content of plants.

In the work of Maiwald (4) the reduction of the content of chlorophyll under the influence of potassium salts has been sharply made apparent in potatoes. Maiwald considers the paler color of the leaves as an incipient chlorosis called forth by the inactivation of iron in the plants by superfluously accumulated ions of potassium and chlorine. The experiments of Liesegang (6) with potatoes have confirmed, as to essentials, the observations of Maiwald on the sharp action of the chlorides. Rémy (6) advances an opinion as to the possibility of the influence of the ratio of green and yellow pigments on color. Maiwald (5), in his controversy with Rémy on the change of ratio of green and yellow pigments, alludes to the work of Deuber (1), who found that changes in the content of carotinoids in the soy bean advances parallel with the changes in the content of chlorophyll, and that therefore the ratio of yellow and green pigments remains constant.

Dhein (2) was the first to note that, when studying potassium salts, it is necessary to take into account the fact that they increase the amount of water in plants. He distinguishes the "apparent" content of chlorophyll (the content in a unit of fresh leaves which determines the color of the leaf) from the "true" content in a unit of dry substance, which provides the possibility of judging the formation of chlorophyll. Dhein has observed an actual reduction of the amount of chlorophyll only with large doses of KCl.

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The purpose of our investigation was to determine whether the paler color of leaves is conditioned in the case of large doses of chlorides by an *apparent* or by an *actual* reduction of the content of chlorophyll; to determine what is the dynamics of the chlorophyll content in plants fertilized and unfertilized with salts containing chlorine. Determinations were made of chlorophyll content in leaves at different stages of development of the plants.

Experimentation

The work was performed during the summer of 1933 with an early variety of potato, Epicure. The experimental plants were grown in pot and in field conditions. Each plot in the field experiments was given a complete fertilizer containing 60 kg. per hectare of N, P_2O_5, and K_2O. The characteristic features of each plot were as follows:

A. (control). Fertilizers were given in the form of precipitates of calcium, KNO_3, and urea; therefore they did not contain any chloride or SO_4.
B. During one year Cl was introduced in the form of KCl and of NaCl.
C. N was provided in the form of NH_4Cl, and K in the form of K_2SO_4.
D. During two years Cl was introduced in the form of KCl and of NH_4Cl.

} Phosphorus was given in the form of superphosphate.

The pot experiment was started in sandy cultures according to the following scheme:

Fertilizer	Per kilogram (Cl^- or $SO_4^=$)	Per pot (Cl^- or $SO_4^=$)
NPK	.....	.....
NPK + Cl	0.210 gm. Cl^-	2.94 gm. Cl^-
NPK + Cl	0.420 gm. Cl^-	5.88 gm. Cl^-
NPK + SO_4	0.288 gm. $SO_4^=$	4.03 gm. $SO_4^=$

As a basis the mixture of Hellriegel was taken. The amounts of Cl^- and $SO_4^=$ are added above those corresponding to Hellriegel's mixture. The forms of salts introduced were varied in a way which made it possible to keep the amount of N, P, and K per pot unchanged.

Sowing of the field plants was carried out on May 29. Complete and simultaneous young growth appeared on July 2; full flowering on July 20.

Samples for determination of chlorophyll were taken at five dates, with three plants per plot, as follows:

Date	Age
1. July 14	13 days (swelling buds)
2. July 22	21 days (flowering)
3. August 1	30 days
4. August 15	45 days
5. August 25	55 days

The sowing in pots was performed on June 9, one plant per pot. The plants were harvested at four dates. Duplicate samples were used.

The average samples taken per plant in order to determine the chlorophyll were collected in the following way: from each top leaflet, or from each top pair of leaflets of a composite leaf, disks of a definite diameter (usually 1.5–2.0 cm.) were cut out with a metal stamp, avoiding the midribs. The determination of water content was carried out in parallel on an equal number of disks of symmetrical disposition.

The chlorophyll was determined colorimetrically, by means of a comparison of acetone extract obtained from ground fresh material with a standard solution prepared according to Guthrie (3) and corresponding to 85 mg. of chlorophyll per liter.

Several analyses were performed with the separation of the green and yellow pigments by saponification with KOH. The amount of green and yellow pigments in these analyses was determined by means of a colorimetric comparison of the latter with standard solutions of Guthrie. However, the values obtained for the yellow pigments cannot be considered correct. The amount of yellow pigments exceeded two or threefold that of the green ones. A more or less rapid determination of yellow pigments still demands a detailed study of the question of standards for comparison, as well as of the previous removal of the colored substances which might hinder determination.

Besides the determination of the water content and amount of chlorophyll in the plants, analyses were also made in parallel on the content of chlorine, and, in part of the material, also on that of nitrogen. It was necessary to determine the influence of various forms of nitrogen fertilizers upon the content of nitrogen in the plants, as it is known that the color greatly depends on the nitrogen content.

The analysis for nitrogen was carried out only on plants of the second date in the field experiment, in which the differences in chlorophyll content were rather sharp, a nearly equal content of nitrogen, 2.80–2.90 per cent., being discovered. It is obvious that the sharp differences observed in the chlorophyll content could not be conditioned by differences in the content of nitrogen.

Results

CROP DATA.—The depressing action of chlorides was more or less sharply expressed in field conditions but hardly displayed at all in the pot cultures.

If the weight of the tubers per plant in the field experiment with NPK may be considered equal to 100, in pots fertilized with chlorides it equals 80–82.

The amount of chlorine in the plants varied according to the dose of fertilizers introduced and to the age of the plants.

TABLE I

CONTENT OF CHLORINE IN POTATO LEAVES IN PERCENTAGE (DRY SUBSTANCE)

SCHEME	JULY 14	AUGUST 1	AUGUST 15
	%	%	%
NPK	0.06	0.32	0.04
NPK + Cl 1 year	1.62	1.80	2.22
NPK + Cl + SO_4	1.29	1.54	1.94

The addition of chlorides was rather sharply expressed in the increase of water content in plants. In table II are quoted data on the content of water in the whole plant (stems and leaves), and in the leaves separately (in the sample which was taken in parallel with the determination of chlorophyll).

TABLE II

AMOUNT OF WATER IN PLANTS OF THE FIELD EXPERIMENT IN PERCENTAGE (FRESH SUBSTANCE)

SCHEME	JULY 14 WHOLE PLANT	JULY 22 LEAVES	AUGUST 1		AUGUST 15		AUG. 25
			WHOLE PLANT	LEAVES	WHOLE PLANT	LEAVES	LEAVES
	%	%	%	%	%	%	%
NPK	89.1	81.5	83.7	83.7	84.9	83.2	85.3
NPK + Cl 1 year	92.2	83.7	88.7	88.5	87.6	86.7	86.0
NPK + Cl 2 years	.....	83.5	86.4	88.0	.....	86.3	87.8
NPK + Cl + SO_4	92.1	83.4	86.5	88.0	87.3	87.1	86.1

In the plants of pot experiments no noticeable differences in the water content could be observed.

COLOR OF PLANTS.—In the field experiment, on plots which had received high doses of chlorides the plants began, approximately at the age of 18–22 days, to exhibit a paler color. This difference became still more evident as the plants grew to maturity. In the pot experiments no differences in color were noticeable to the eye.

TABLE III

CONTENT OF CHLOROPHYLL IN PLANTS OF FIELD EXPERIMENT

SCHEME	JULY 14		JULY 22			AUGUST 1			AUGUST 15			AUGUST 25		
	FRESH WEIGHT	Mg. PER CM.^{2}	FRESH WEIGHT	DRY WEIGHT	Mg. PER CM.^{2}	FRESH WEIGHT	DRY WEIGHT	Mg. PER CM.^{2}	FRESH WEIGHT	DRY WEIGHT	Mg. PER CM.^{2}	FRESH WEIGHT	DRY WEIGHT	Mg. PER CM.^{2}
	%	*mg.*	%	%	*mg.*	%	%	*mg.*	%	%	*mg.*	%	%	*mg.*
NPK	0.18	0.051	0.31	1.67	0.083	0.22	1.48	0.061	0.25	1.43	0.071	0.23	1.54	0.064
NPK + Cl 1 year	0.17	0.047	0.21	1.30	0.062	0.18	1.55	0.054	0.22	1.67	0.068	0.16	1.16	0.049
NPK + Cl 2 years	.....	.....	0.21	1.28	0.061	0.18	1.50	0.060	0.20	1.46	0.056	0.11	0.22	0.032
NPK + Cl + SO_4	.....	.....	0.25	1.53	0.067	0.16	1.36	0.051	0.19	1.58	0.057	0.22	1.53	0.059

CONTENT OF CHLOROPHYLL.—The chlorophyll content in plants is given (table III) in percentage for fresh and dry substance, and in mg. per cm.^{2} of surface area (each figure represents the mean value of three single determinations).

The content of chlorophyll per unit of fresh weight and of surface is much lower in chloride variants than in controls, which regularly recurs on all the five dates. The content of chlorophyll in dry substance is sharply reduced toward the end of vegetation, August 25, and somewhat less reduced, yet sharply lowered, in July. On both the other dates the chloride variants even exceeded the controls. The data for August 1 and 15 are interesting. Notwithstanding the greater content of chlorophyll per unit of dry substance in plants from plots fertilized with chlorides, the color is much lighter than that of the controls. This is seen both from the values for fresh substance of the plant and from the values per unit of surface. In both cases there is an apparent reduction of chlorophyll which is due to the increase of water content in the leaves.

As to the dynamics of the chlorophyll content in variously fertilized plants during the vegetative period, the content of chlorophyll in the dry substance was more or less stable both in controls and in plants having received small doses of chlorides plus sulphates, whereas in plants which had received high doses of chlorides there was noted as early as August 25 a sharp reduction of chlorophyll content in the dry as well as in the fresh substance of the plant. The fluctuations in the percentage of chlorophyll content in the fresh substance, at different dates, were to a great extent conditioned by the change in the water content of the plant. From figure 1 it is seen that the maximum chlorophyll content coincides with the minimum of water content and *vice versa*.

The determination of the yellow pigments, as mentioned previously, can hardly be considered sufficiently accurate. However, these few tentative investigations allow the conclusion that the ratio of the green and yellow pigments was more or less constant, and therefore the different colors of the leaves did not depend on the change in the ratio of green to yellow pigments.

The percentage of chlorophyll in the plants of pot cultures offered a somewhat different picture from that of the field experiment. The actual reduction of chlorophyll content in the fresh and dry substance is here noticeable only at the last date. On the other dates the chlorophyll content in plants fertilized with chlorides, expressed on the dry and fresh weight basis and on the fresh leaf surface, was equal to that in the controls and in many cases it even exceeded the latter.

It is of interest to note the differences observed in our experiments between plants that have been grown under field conditions, and those

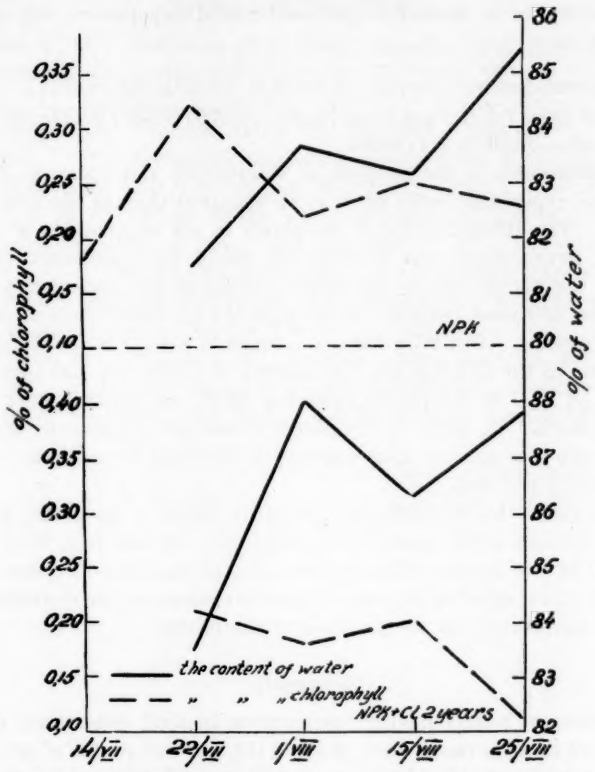

FIG. 1. Content of chlorophyll and water in percentage (fresh matter) in leaves of potato plant at different stages of growth; field experiment.

that were grown in greenhouse conditions (the pot cultures in vessels of MITSCHERLICH's type stood during the summer under a metal wire net.

First of all the plants of the pot experiments contained much less chlorophyll than the plants of the field experiments. We shall cite several examples where the plants assorted are of the same age.

Variant NPK. Pot experiment, age of plants 26 days, percentage of chlorophyll of fresh substance 0.11 per cent.

Same variant. Field experiment, age of plants 21 days, percentage of chlorophyll to fresh substance 0.31 per cent.

Pot experiment—44 days, 0.12 per cent.; field experiment—45 days, 0.25 per cent.

Pot experiment—59 days, 0.12 per cent.; field experiment—55 days, 0.23 per cent.

If expressed as mg. per cm.^{2} of surface, we obtained results at the above mentioned ages, for the pot experiments—0.026; 0.032; 0.024; for the field experiments—0.083; 0.071; 0.064.

The differences in the contents of chlorophyll and water in the plants of the field experiment were much more apparent than in the plants of pot cultures. The different color of the plants in our experiments at the early stages of development was conditioned chiefly by a different content of water in the plants. But, just what conditions the increased content of water (the increased entrance of chloride ion or other factors) is difficult to say at present. In field plants, in a series of cases, parallel to the rise of the content of the chloride ion, the content of potassium also increased 1.7 per cent. of K_2O in the plants receiving NPK, and 2.6 per cent. in those receiving NPK + Cl, while in the plants of the pot experiment the content of potassium was more or less constant in different variants, in both cases nearly 2.0–2.1 per cent. K_2O.

It is possible that the different content of water in the plants was called forth by changes in the content of potassium. At any rate, work must be continued on the interpretation of the action of chlorides in order to isolate the action of the chloride ion itself from the changes in the nutrition régime of plants conditioned by the presence of the latter.

Summary

1. Leaves of plants having been grown in field conditions, contained two to three times as much chlorophyll as the leaves of plants of pot cultures.

2. The action of chlorides on the total crop and on the content of water and chlorophyll in the plants was more sharply expressed on the plants of field experiments than on those of the pot experiments.

3. The lower content of chlorophyll in the fresh leaves of plants on plots having received high doses of fertilizers containing chlorides at early stages of development was conditioned by an increased content of water in those plants.

4. The actual reduction of the chlorophyll content (percentage dry substance) in plants rich in chlorine took place only at the end of the vegetative period of the plants.

The writers express their acknowledgment to Professor A. V. Blagowe-schensky for his valuable guidance in the course of this work.

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THE CHLOROLYTIC ACTION OF SERUM

MARY ELMORE SAUER

(WITH ONE FIGURE)

Introduction

In studying the effect of serum on euglenas, the writer had previously observed (ELMORE, 4) that whenever euglenas killed in various ways were placed in contact with serum, the latter became tinged with green. It was shown that this diffusion of chlorophyll resulted from the action of serum alone on the euglenas, without the intervention of any third factor such as plant or animal complement. The phenomenon was referred to as chlorolysis, a term introduced by SHERWOOD (7) to describe a release of pigment from chloroplasts, which occurred only in the presence of both serum and a plant extract.

The present paper reports a further study of the chlorolysis resulting from contact of chlorophyll-containing bodies with serum.

Material and methods

The work described here is based upon pure cultures, free from bacteria, of the following unicellular chlorophyll-bearing organisms:

Euglena gracilis Klebs.

Protosiphon botryoides Klebs.

Two algae whose identity is uncertain but which are probably undescribed species of *Chlorella*:

No. 5. Shape of cells usually oval; size variable, average 6 by 9 μ; many oil droplets in cytoplasm; chloroplast cup-shaped; luxuriant growth.

No. 11. Large cells, 15 μ in diameter, within which many non-motile daughter cells form; clathrate chloroplast; luxuriant growth.

Suspensions of chloroplasts of several higher plants were also used.

The euglenas were isolated by the writer and grown in a broth of peptone and inorganic salts, from which they were centrifuged for use. The other forms were obtained through the courtesy of Dr. FRANK B. WANN, and cultivated upon an agar medium containing inorganic salts and dextrose.

When not otherwise stated, chlorolysis was obtained by adding 0.5 cc. of a 10 per cent. suspension of *Euglena gracilis,* previously heated for 10 minutes at 45° C., to 0.5 cc. of a 1:5 dilution of human serum. After an hour's incubation at 37° C., the cells have settled, leaving a deep blue-green supernatant fluid.

Investigation

Serum factor

Chlorolysis occurred from every serum experimented with, whether fresh or inactivated, recently drawn or preserved for many weeks. Dog, guinea pig, and numerous samples of human and rabbit serum were used. No attempt was made to collect the blood in the fasting state. The amount of chlorolysis varied with different serums, as will be discussed below.

The writer (**4**) reported previously that the amount of chlorolysis obtained with rabbit serum is not increased by immunization. In agreement with Sherwood (**7**), common plant extracts possess no chlorolytic property such as that found for serum.

The total amount of chlorophyll that diffuses out during chlorolysis must depend primarily upon the comparative solubility of the chlorophyll in the medium within and without the cell. Inasmuch as chlorophyll is lipoid soluble, it was thought probable that the lipoid of the serum was the solvent concerned in the medium surrounding the cell. The following findings tend to support this view.

Bloor (**1**) states that the values for total lipoid in blood show wide variations in different species and to a less extent in individuals of the same species. Since human serum seemed consistently to have a greater chlorolytic effect than rabbit serum, a series was set up better to determine quantitative variations. Table I shows that the chlorolytic power of the serums used decreases in the following order: dog, human, rabbit, guinea pig. Bloor (**1**) gives 0.490 as the average total lipoid by weight per 100 cc.

TABLE I

Comparative chlorolytic power of serums*

Species	Serum dilution				
	Undiluted	1:5	1:10	1:25	1:50
Dog	4	4	4	3	1
Human	4	4	4	2	1
Rabbit	4	4	3	1	0
Guinea pig	....	3	2	1	0

* 0.5 cc. of a 12% suspension of *Euglena gracilis* in 0.9% sodium chloride solution was added to 0.5 cc. of the serum dilution indicated. The suspension had been heated in a water bath at 50° C. for 10 minutes. Incubation period was 1½ hours at 37° C., followed by thorough shaking, then 24 hours in refrigerator. Figures represent arbitrary degrees of chlorolysis.

plasma for the dog without food for 24 hours. MATHEWS (**6**, p. 486) gives 0.1 to 0.3 per cent. as the normal figure for fat in human serum. BÜSSEN's (**3**) range for normal rabbits on normal feedings, taken at different times of the day and determined by Bang's micromethod, is 0.057 to 0.063 per cent. total fat. Figures for guinea pig were not available.

The effect of increased fat in the serum following excessive fat ingestion was studied. A dog was bled nine hours after a carbohydrate and protein meal and again three hours after a meal rich in fat. The chlorolysis resulting from the latter serum was much greater. The tests with the two serums were carried out at the same time and the tubes read in a comparator. Also, fatty human serums had a greater chlorolytic effect than other normal samples.

Considerable chlorolysis resulted from an emulsion containing the lipoids extracted from serum by alcohol and ether, following the method of BLOOR, PELKAN, and ALLEN (**2**). The filtrate was evaporated, and sufficient physiological saline solution added to make the volume equal to that of the original serum. From these experiments, it appears that the amount of the chlorolysis varies with the lipoid content of the serum used.

CHLOROPLAST FACTOR

Cell death was a prerequisite of the euglena chlorolysis mentioned above. No chlorolysis resulted when unicellular algae, which had previously been exposed to the temperature found to be lethal to the euglenas (10 minutes at 45° C.), were incubated with serum. Since it was not certain that the algae were killed by this treatment, their thermal death points were determined. These are given in table II.

Heavy suspensions of these organisms were prepared in 0.9 per cent. sodium chloride solution. Each was heated to its thermal death point and

TABLE II

THERMAL DEATH POINT AND LIPOID CONTENT OF ORGANISMS USED

ORGANISM	THERMAL DEATH POINT	LIPOIDS BASED ON DRY WEIGHT*
		%
Euglena gracilis (normal green culture)	45° C.	10.4
Chlorella sp. no. 5	48° C.	9.9
Chlorella sp. no. 11	52° C.	2.7
Protosiphon botryoides	50° C.	.....

* Extraction with redistilled petroleum ether in cold. Result is mean of two trials.

incubated with an equal quantity of serum, but again chlorolysis occurred only in the case of the euglenas. However, when *Chlorella* sp. no. 5, whose thermal death point is 48° C., was heated for 30 minutes at 56°–60° C., chlorolysis, although less in quantity, was obtained just as with the euglenas heated only to their thermal death point. Evidently, with this species of *Chlorella* it is necessary to heat to a temperature somewhat above the thermal death point in order to obtain chlorolysis. However, with the other two algae, *Chlorella* sp. no. 11 and *Protosiphon botryoides,* chlorolysis could not be obtained even after heating at 75°–80° C. for one-half hour. With the euglenas the amount of chlorolysis was only slightly greater with organisms heated for 10 mniutes at 50° C. than with those heated at 45° C.

In addition to the unicellular organisms just mentioned, similar tests were made with the chloroplasts contained in finely ground suspensions of leaves of alfalfa (*Medicago sativa*), red clover (*Trifolium pratense*), and the common broad-leaved plantain (*Plantago major*). Heavy washed suspensions of ground leaves (10 to 20 per cent.) were used. Chlorolysis occurred with serum, but the color of the supernatant fluid was slight as compared with the very intense blue-green color resulting when a heat-killed 10 per cent. euglena suspension was incubated with an equal or smaller quantity of serum. Serum was necessary for chlorolysis, as shown by the fact that the control tube containing 0.9 per cent. sodium chloride solution in place of serum in every instance remained perfectly colorless.

Why the same serum releases different amounts of chlorophyll from different types of cells requires further comment. The condition of the chlorophyll within the living chloroplast is debated. ZIRKLE (**10**) gives evidence that it is not in a lipoid solution, as has been held. Whatever the condition of the chlorophyll within the dead and altered cells with which we are dealing, chlorolysis appears to be a process by which the chlorophyll concentration reaches an equilibrium between the cell and the surrounding serum. The total amount released varies with conditions under which equilibrium is reached. Inasmuch as lipoids are present in all cells, it was thought of interest to determine whether the amount is greater in the cells releasing more chlorophyll. Reference to table II shows that this is actually the case. *Chlorella* sp. no. 11 contains only a negligible amount of lipoid as compared with *Euglena gracilis* and *Chlorella* sp. no. 5. It will be recalled that chlorolysis resulted from the latter organisms but that it could not be obtained with the former. The lipoid content of *Protosiphon botryoides* could not be determined, owing to its penetrating growth, which made separation from the agar difficult. JACOBS (**5**) warns against drawing conclusions from work with plant cells where the cellulose cell wall may be a complicating factor. This may account for the greater ease of chlorolysis in euglenas than in unicellular algae with similar lipoid content.

These considerations give weight to the view that chlorolysis is a process by which the chlorophyll concentration reaches an equilibrium between the lipoid within the cell and that of the surrounding serum.

Analysis of resulting supernatant fluid

The absorption spectrum of the supernatant fluid from euglena "chlorolysis" was compared with that of an alcoholic extract of fresh euglenas, using a spectroscope provided with a comparison prism. The two spectra were identical. This indicates that the chlorolyzed pigments are in true solution, for in colloidal solutions of chlorophyll the absorption bands are shifted to the red end of the spectrum as compared with those of a true solution (**8**, pp. 348–349).

Rate of chlorolysis

The above work was concerned merely with the total amount of chlorophyll that diffuses out within a definite period of time. While it has been shown (**4**) that the amount of chlorolysis is not greater for immune than for normal serum, the possibility remained that immune serum might bring about more rapid chlorolysis than normal serum. Preliminary experiments showed that some chlorophyll was transferred to the serum within a few seconds. In order to study the rate at which diffusion goes on, a method was devised by means of which the process is slowed down sufficiently to record the early changes.

The method consists of repeated filtrations of the serum through a pad of asbestos fibers on which euglenas or other cells are held, in a Gooch crucible. With the suction applied, 2 cc. of a 1:10 dilution of serum in physiological saline solution were added. The filtrate was collected in a test tube, and the time recorded in seconds from the moment of adding the serum to the moment of release of suction when filtration was complete. After each filtration the filtrate was quickly matched in a comparator with a standard tube, and the filtration was then repeated.

The amount of chlorolysis after successive filtrations was determined by comparison with a series of standard solutions. Standards prepared from an alcoholic extract of euglenas were too yellow, while extracts of chlorophyll alone were too blue for satisfactory comparison. By the method of Thatcher (**9**, pp. 113–114) the chlorophylls and yellow pigments were separated, and half of the yellow pigments was put back with the chlorophyll. This gave color standards which closely matched in color the solution obtained by euglena chlorolysis. The standard solution was brought by evaporation to a volume representing a 3 per cent. suspension of the original cells from which it was prepared. Using 50 per cent. methyl alcohol as a diluent, a series of concentrations was made, based upon this

standard pigment solution, each successive tube decreasing by approximately 20 per cent.

The results of this study are shown in the accompanying diagram (fig. 1). The rate of chlorolysis is seen to be no greater with immune than

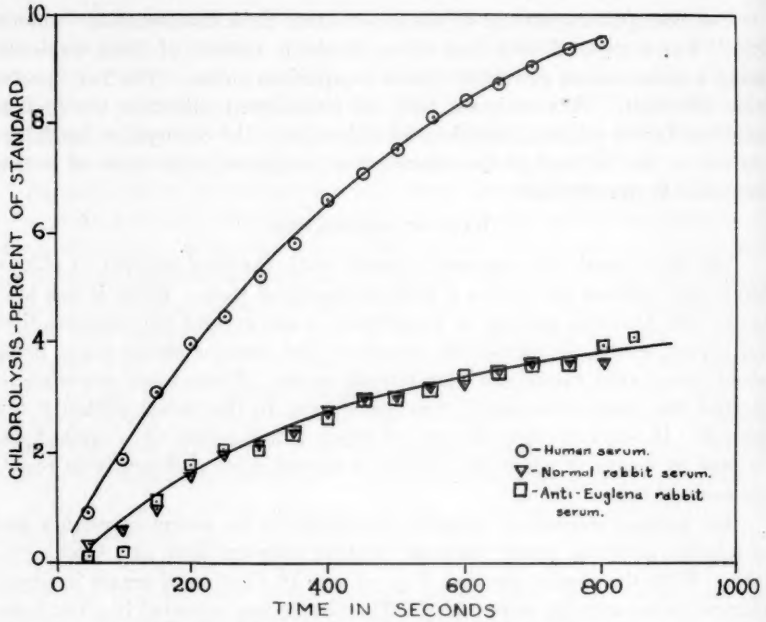

FIG. 1. Rate of chlorolysis with different serums.

with normal rabbit serum. With human serum the diffusion is more rapid than with rabbit serum.

Discussion

The chlorolysis described above results from the action of serum alone. The fact that it is not increased by the addition of plant extract distinguishes it from that described by SHERWOOD (7). SHERWOOD did not observe chlorolysis with serum alone, probably owing to the more dilute chloroplast suspension with which he worked. In repeated attempts to duplicate his results, the phenomenon he described was not observed, an incubation period up to four hours producing no change. However, even after one hour of incubation a blue-green color in the control tube with serum and chloroplasts alone was always detectable if a sufficiently heavy chloroplast suspension was used.

If chlorolysis were more rapid with immune than with normal serum, it would indicate that the immune serum had increased the permeability of the cell to its chlorophyll. That this is not the case is shown by figure 1. The rate of chlorolysis, as well as the amount of chlorolysis, varies with the lipoid content of the serum, but no effect of immunization upon chlorolysis has been found.

The theory is offered that the cell death or further treatment preliminary to application of serum enables some chlorophyll to diffuse from the chloroplasts into the lipoids of the cell. Upon contact with the serum, chlorophyll passes to the serum lipoids up to the point of equilibrium between cell contents and serum.

That mere cell death is a sufficient change to enable chlorolysis to occur with euglenas, that heating somewhat above the thermal death point is necessary for the same result with certain algae, and that others are entirely resistant to the serum, indicate some fundamental differences in the microchemical structure of these cells. The rôle of lipoids and of a cellulose cell wall have been considered.

Conclusions

Serum causes a liberation of the chlorophyll from many chlorophyll-containing cells or chloroplasts, if these have been sufficiently altered by heat or contact with strong serum. This action is associated with the lipoid content of the serum, and appears to require the presence of lipoids in the cell. Immune serum does not increase the permeability of the cell to chlorophyll.

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ANALYSIS OF OSMOTIC RELATIONS BY EXTENDING THE SIMPLIFIED METHOD

CHARLES J. LYON

Students and teachers of plant physiology find in the water relations of individual cells an interesting challenge to their ability to interpret the movements of water on the basis of known laws of physical chemistry. The elementary student usually concentrates on the phenomenon of plasmolysis which shows the presence of a semipermeable membrane and a limited concentration of osmotically active solutes. When the problem is considered with more concern for the actual conditions which prevail when the cells are a part of intact organs, the probable variations in water and solute content, pressures potential or active, and the resulting effects on cell volumes are realized. Chiefly as an outgrowth of the work of URSPRUNG and his colleagues, much thought has been given in recent years to the exact relationships between these variables, but the contribution to the routine instruction of students through laboratory experiments has been relatively small. There has also been an unfortunate development of conflicting terminology which has somewhat handicapped the average student of the subject.

Aside from plasmolytic tests, the one new type of experiment which seems to have been adopted generally is that apparently devised by PRINGSHEIM (**3**), based on the "simplified method" of URSPRUNG (**4**) for measuring the "suction force" (*Saugkraft*) of cells as they are held in plant tissues. As a classroom experiment, uniform blocks or strips of a tissue are immersed for some time in various concentrations of a sugar solution. The strips are measured macroscopically for length before and after immersion; the concentration of solution in which the strip neither lengthens nor shortens is taken as a measure of the mean net osmotic pressure of the cells in their normal state as a part of a tissue or organ. The potato tuber is the usual source of these strips because a large tuber will provide enough tissue for a long series of concentrations.

Even when the student has noted the results of this experiment and computed the net osmotic pressure for the tuber used, this figure can be compared only with the water attracting power *at incipient plasmolysis* of entirely different cells, commonly those of an alga or a leaf epidermis. The value of the important concept of changing osmotic concentrations and net potential pressures with variations in volume and turgor pressure is largely lost because there has been no demonstration of these changes within a single tissue. It might be possible to have some students make the microscopic measurements of changing cell volumes after the procedure developed by URSPRUNG (**1, 6**), but the time and skill are usually not available in courses

for undergraduates; hence the instruction on this phase of cellular physiology is chiefly verbal.

In a search for an experimental method by which students might develop the complete picture of changing values within a single tissue, the tissue strip experiment was considered for its possibilities of extension. As a means of indicating the net osmotic pressure of cells under normal conditions, the method considers the result obtained to be the mean value for all the cells in the strip used. However, in principle the method considers the strip as one large cell and the longest dimension is measured with a rule as the length of a cell would be measured with an ocular micrometer in a compound microscope. To carry the experiment further than the mere determination of the net osmotic pressure of the tissue in the condition in which it is removed from the organ or plant, it would be necessary to note the length of this gigantic cell when saturated with water and again when shrunken the most possible at the concentrated end of the series of solutions. In passing toward this end of the series, the first solution which gave the maximum shrinkage would be isosmotic with the cell contents at incipient plasmolysis. In other words, its osmotic pressure would be a measure of the water attraction of the cell contents at its smallest volume short of actual plasmolysis.

This extended form of the experiment would therefore give strip length at incipient plasmolysis, in the normal phase and in the saturated condition with the osmotic pressure of both cell and cell contents at incipient plasmolysis known from the molal concentration of the corresponding external solution. The mean net osmotic pressure of the cells under normal conditions is also known from the molal concentration of the external solution which allows neither shrinkage nor enlargement, but this value can better be used as a check on the accuracy of the measurements, since it can also be calculated by URSPRUNG'S method (**5, 6**) for individual cells.

These calculations are normally made on the basis of the incipient osmotic pressure of the cell contents and the three cell volumes at incipient plasmolysis, normal, and saturated conditions respectively. To obtain the data for calculating these volumes, it would be necessary to measure also the average cross sectional area of the strip at each of these three critical points. This is admittedly the best possible practice, but our studies indicate that very satisfactory results can be obtained with firm tubers and uniformly cut strips of tissue, merely by taking the length of strip as an index of its volume (at each of the three critical points) for all the necessary calculations. In fact, the accuracy of each set of results will be tested by the comparison between observed and calculated data for net osmotic pressure of the cell in the normal phase, since the calculations are based partly on other observations.

Although it is feasible to cut uniform strips of fairly homogeneous tissue

from many plants, as MOLZ (**2**) and others have done in the analysis of normal net osmotic pressure by the simplified method, the requirements for a classroom demonstration of relations at all degrees of water supply do not permit such a wide range of choice. Experienced workers may deal successfully with tendencies to twist and curl and they will likewise avoid such mistakes as the inclusion of large veins in working with leaf parenchyma. Inexperienced students need to use long strips of tissue which they can cut with ease and measure with accuracy. In all cases it is desirable that the cells show an appreciable change in volume between plasmolysis and saturation. The following data and descriptions of procedure are therefore based on the use of the potato tuber and ordinary laboratory equipment. The same method will apply to other homogeneous material and more exact measurements with better equipment.

Typical results and the necessary calculations for two sets of data are given to show the operations and procedure (table I). Actually these were the results in the first two tests made of the method here suggested, with measurements of lengths made by elementary students.

TABLE I

MOLAL SOLS. OF SUCROSE	OSMOTIC PRESSURE IN ATM. AT 20° C.	LENGTHS			
		TEST A		TEST B	
		START	24 HOURS LATER	START	LATER 24 HOURS
		mm.	*mm.*	*mm.*	*mm.*
(Water)	.....	70	73.8	70	72.1
0.2 M	5.29	70	70.8	70	70
0.3	8.13	70	69	70	69
0.4	11.10	70	67.8	70	67.7
0.5	14.3	70	64.2	70	66
0.6	17.8	70	62.4	70	64
0.7	21.5	70	62	70	62.8
0.8	25.5	70	62	70	62

The strips were taken from a single large tuber for each test. They were all cut from the same zone (the cortex), parallel to the long axis, by means of a cork borer which removed cores about ¼ inch in diameter. These cores were rinsed in distilled water to remove excess starch grains and from the mid-section of each was cut a cylinder exactly 70 mm. long. Without allowing them to dry, these cylindrical strips were immersed in the sugar solutions, one to each contained in a stoppered test tube of about 25 cc. capacity. The next measurements were made the following day, although it may be possible to obtain the same results by allowing only 3 or 4 hours between measurements.

Since the accuracy of the method depends largely on precise measurements of length, these were made with a rule marked in 0.2 mm. A wide-field binocular dissecting microscope was used to take the readings at both ends of the strip while the rule was held firmly against it. Much time can be saved in starting with cores of equal length (not necessarily 70 mm.) if some device is rigged to hold safety razor blades firmly at such a distance apart.

The calculations were made according to URSPRUNG'S method for single cells (**5, 6**), the osmotic relations of which are expressed by his equation

$$S_s = S_1 - W.$$

Written in terms of osmotic pressure, this equation becomes

net pressure = cell contents pressure − wall pressure.

For purposes such as given in table II and calculations, this statement can be abbreviated to read

$$N = C - W.$$

The method presupposes (*a*) that the contents pressure (C) of each cell varies inversely with the volume and (*b*) that the wall pressure (W) varies directly with the volume. Both the critical data and necessary calculations are shown for test A, while for test B the calculations are omitted since the results are obtained in the same way.

TABLE II

	Test A			Test B		
	Incipient plasmolysis i	Normal n	Saturated s	Incipient plasmolysis i	Normal n	Saturated s
Volume	62	70	73.8	62	70	72.1
C (in atm.)	21.5	X = 19.04	X′ = 18.06	25.5	X = 22.59	X′ = 21.93
W " "	0	Y = 12.24	X′ = 18.06	0	Y = 17.37	X′ = 21.93
N " "	21.5	Z = 6.80	0	25.5	Z = 5.22	0
	Observed Z = 6.53			Observed Z = 5.29		

$$X = C_n = \frac{C_i \cdot V_i}{V_n} = \frac{21.5 \cdot 62}{70} = 19.04$$

$$X' = C_s = \frac{C_i \cdot V_i}{V_s} = \frac{21.5 \cdot 62}{73.8} = 18.06$$

(= W_s also since N is 0 when cells are saturated with water)

$$Y = W_n = \frac{W_s(V_n - V_i)}{V_s - V_i} = \frac{18.06 \cdot 8}{11.8} = 12.24$$

Z = X − Y = 19.04 − 12.24 = 6.80 (calculated value)

Z = osmotic pressure of 0.244 M sol. = 6.53 (observed value)
(0.244 obtained by interpolation in table I for solution giving no change in volume of cells)

From these data for two tests, the student has obtained a well defined picture of water relations in a certain tissue. There is also striking evidence for the following deductions:

1. Tuber A was made up of cells containing relatively less solutes than those of tuber B, since the osmotic pressure of its cell contents (C) is always smaller.

2. In spite of this condition, the cells in tuber A in their normal condition (in February) had a greater net osmotic pressure (N) and swelled more when immersed in pure water.

3. However, largely because of the greater solute concentration in tuber B, the turgor presure of its cells (measured by the opposing wall pressure) was greater in both normal and saturated condition than the corresponding pressures of tuber A.

4. Tuber A may have been a younger tuber than B in point of maturity, since its solute content was less and since its cell walls seemed to have less mechanical resistance to even less internal pressure, as indicated by the greater swelling in A before equilibrium was attained in water.

If results comparable to these for the potato tuber are desired from other tissues, there are various fleshy fruits, roots, and stems from which long strips can be obtained, suitable for macroscopic measurements. Tests of such points as degree of maturity, degree of desiccation, and total solutes might well be made with such organs for their possible commercial value, since the necessary measurements could be made easily and quickly in field or warehouse without elaborate equipment or extensive training of the worker.

It will be noted by those familiar with the term *suction force* (also known to some as suction tension), that it has been avoided here in favor of its equivalent of *net osmotic pressure* wherever it applies to the water attracting action of a cell or tissue. It is recognized that the attraction between water and certain parts of a plant cell is due to forces of imbibition, but this component of the total attraction of water to the cell can safely be measured with the true osmotic forces, and the total expressed in the usual units of osmotic pressure without denying the action of imbibition in the process.

If, for purposes of making vivid the nature of water movement as the result of an attraction expressed as osmotic pressure, it becomes desirable to liken the movement to that induced by a suction pump, the term suction force can readily be used as an added alternative for net osmotic pressure in oral or informal discussions. It is unfortunate that the word pressure does not at first thought suggest a power to draw something unto itself, but there is no more real need to replace it completely by a more vivid word than there is to change the signs plus and minus with respect to high and low potentials in a conductor of electricity, just because the flow of electrons is actually

from low potential to high potential and not from plus to minus as we conventionally speak of the flow of an electric current.

Summary

Slender, cylindrical strips of homogeneous tissue, such as that of the potato tuber, can be treated like single cells in the complete analysis and demonstration of osmotic relations in a healthy plant tissue. In its simplest form, this extension of the "simplified method" is available for elementary instruction in the subject while at the same time it gives quantitative results in the determination of net osmotic pressures and turgor pressures, in accordance with the modern interpretation of such phenomena.

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ADAPTATION OF THE MICRO–KJELDAHL METHOD FOR THE DETERMINATION OF NITROGEN IN PLANT TISSUES[1]

NEIL W. STUART

Introduction

The micro-Kjeldahl method for nitrogen determination has been widely used in organic chemistry laboratories in recent years. The value of this method, developed through the brilliant researches of PILCH, BANG, PARNAS and WAGNER, PREGL, and coworkers, has justified its application to industrial and clinical problems. Plant physiologists have not generally adopted quantitative micro-methods for nitrogen analysis. The purpose of the present study was to test the applicability of the micro-Kjeldahl method for the determination of nitrogen in plant materials.

Review of literature

Numerous modifications of micro or semi-micro methods have been proposed for the determination of nitrogen in small amounts of material. This paper is not concerned with clinical methods developed for specialized purposes.

PREGL (9) has reviewed the early work on micro-determination of nitrogen by the Kjeldahl method and gives detailed directions for the analytical procedure.

MOTHES (7) employed the PREGL micro-Kjeldahl method in a study of nitrogen metabolism in plants. He states that the errors of the method were less than 1 per cent. but gives very few details of how the analyses were performed.

HARTLEY (5) has described a dilution method for micro-Kjeldahl determinations when no microchemical balance is available. In this method a certain number of drops of partially digested acid solution are taken for analysis so the results can not be strictly accurate.

ALLEN (1) modified the PARNAS and WAGNER (8) distillation apparatus used by PREGL, permitting the analysis of larger samples.

CAVETT (2) developed a semi-micro-Kjeldahl method to analyze small quantities of nitrogen in large volumes of liquid. He claims for the method and apparatus an accuracy of 0.006 mg. of nitrogen when determining from 0.5 mg. to 14 mg. of nitrogen.

MORRIS (6) has reported successful use of a slight modification of the CAVETT method in plant metabolism studies.

EMMERT (4) has reported a rapid semi-micro method for determining

[1] Published with the approval of the Director of the University of Maryland Agricultural Experiment Station.

nitrogen in plants. This method requires that the sample be oxidized with sodium chlorate and H_2SO_4. The nitrogen is estimated by the phenol-disulphonic acid colorimetric method.

DONEEN (3) has described a micro-method for organic and nitrate nitrogen through the use of salicylic acid and perchloric acid. The nitrogen is estimated colorimetrically after Nesslerization.

Materials and methods

PREPARATION OF SAMPLES.—Fresh plant tissues used in this study were first passed through a Nixtamal mill. The method of extracting the non-protein nitrogen has been described in a previous publication (12). Portions of the macerated tissue were dried at 70° C. The dry tissue was finely ground in a hand mill and stored in small aluminum boxes which were placed in the case housing the microchemical balance. The amount of moisture in this tissue at the time the nitrogen analyses were made was determined by drying 1-gm. portions at 80° C. and a pressure of 3 to 4 cm.

MACRO-METHODS.—Total nitrogen in the dry tissue and in the non-protein extracts was determined by the Gunning modification of the Kjeldahl method not modified for nitrates. Basic nitrogen, precipitated by phosphotungstic acid, was determined as described in a previous publication (12). Amide nitrogen was determined by hydrolysis of aliquots of the non-protein extract with 10 per cent. H_2SO_4 followed by aeration of the alkaline solution for 16 hours by the SESSIONS and SHIVE method (11).

MICRO-METHODS.—Total nitrogen in the dry tissue was determined by the micro-Kjeldahl method described by PREGL (9). All weighings were made on a Kuhlmann microchemical balance. About 25 mg. of dry plant tissue were weighed in a Coors porcelain boat. Boat and contents were transferred to a microdigestion tube and digested with 1 ml. of low nitrogen concentrated H_2SO_4 and a few mg. of 3:1 $CuSO_4$–K_2SO_4 mixture. When the solution was partially clear, two or three drops of Merck's reagent Superoxol (30 per cent. hydrogen peroxide) were added and the digestion completed. The acid digest was diluted with 1 ml. of ammonia-free water and washed into the distillation apparatus.[2]

After adding 7 ml. of 30 per cent. NaOH containing 5 per cent. $Na_2S_2O_3$ the mixture was distilled with steam for 5 minutes. The ammonia was collected in 10 ml. of 0.01 N H_2SO_4. Distillates were boiled and titrated with 0.01 N NaOH to a methyl red endpoint. The indicator, introduced by means of a glass thread, was prepared by adding an excess of methyl red to 0.1 N NaOH. Acid and base were measured in 10-ml. microburettes. The apparatus was steamed for 30 minutes before each series of determinations. Blank determinations amounted to 0.05 to 0.06 ml. of 0.01 N NaOH.

[2] Manufactured by the firm of Paul Haack, Vienna.

The nitrogen content of 2-ml. aliquots of the non-protein extracts was determined by the micro-Kjeldahl method. The non-protein extracts were first concentrated *in vacuo* to a volume such that 2 ml. contained 0.3 to 0.5 mg. or more of nitrogen.

Basic nitrogen precipitated by phosphotungstic acid was determined as the difference between non-protein and non-basic nitrogen. The filtrate from the basic nitrogen was neutralized with NaOH, acidified with acetic acid, and concentrated *in vacuo* to the original volume of the aliquot precipitated with phosphotungstic acid. Aliquots of 2 ml. of this basic-free solution were analyzed for nitrogen content by the micro-Kjeldahl method.

Aliquots of 5 ml. of the non-protein extracts were hydrolyzed in micro-digestion tubes with 0.3 ml. of concentrated H_2SO_4 under reflux condensers for 2.5 hours. The solutions were washed into the distillation apparatus and distilled with 2.4 ml. of 30 per cent. NaOH for 5 minutes.

Experimental results

ANALYSIS OF AMINO ACIDS.—The accuracy of the apparatus and method was first tested by determining the nitrogen content of several commercial preparations of amino acids. The results are shown in table I.

TABLE I

NITROGEN CONTENT OF AMINO ACIDS AS DETERMINED BY THE MICRO-KJELDAHL METHOD

AMINO ACID	WT. OF SAMPLE	PERCENTAGE OF NITROGEN		PERCENTAGE RECOVERY
		FOUND	CALCULATED	
	mg.	%	%	%
Tyrosine	6.940	7.64	7.73	98.84
Tyrosine	8.846	7.70	.....	99.61
Leucine	6.488	10.52	10.68	98.50
Leucine	9.647	10.50	.....	98.31
Cystine	6.987	11.56	11.66	99.14
Cystine	7.960	11.58	.....	99.31
Glycine	4.475	18.60	18.65	99.73

COMPARISON OF MACRO- AND MICRO-METHODS FOR THE DETERMINATION OF NITROGEN IN PLANTS.—Typical results, showing the precision of the micro-Kjeldahl method with reference to the macro-methods, are shown in tables II and III. Each value reported is the mean of duplicate determinations. Both macro- and micro-determinations were made from single samples which were collected at intervals during the year.

Discussion

The micro-Kjeldahl method and apparatus used in this study are reliable and accurate within commonly accepted limits of error as shown by the data in table I.

Plant tissues are usually lower in nitrogen than many substances analyzed in organic chemistry laboratories. Many plant tissues contain less than 3.00 per cent. of total nitrogen in their dry tissue. Accordingly, 0.1 mg. of tissue would contain less than 0.003 mg. of nitrogen. The writer has found it possible to titrate duplicate ammonia distillates to within 0.02 ml. of 0.01 N NaOH. This amount of reagent is equivalent to 0.0028 mg. of nitrogen or about the amount contained in 0.1 mg. of tissue. Under these conditions it is necessary to weigh the sample accurately only to the first decimal of a milligram. This is best accomplished by weighing the

TABLE II

COMPARISON OF MACRO- AND MICRO-METHODS FOR THE DETERMINATION OF TOTAL AND NON-PROTEIN NITROGEN IN PLANT TISSUES

TOTAL NITROGEN EXPRESSED AS PERCENTAGES OF THE DRY WEIGHT AND NON-PROTEIN NITROGEN AS PERCENTAGES OF THE FRESH WEIGHT

PLANT TISSUES	TOTAL NITROGEN		NON-PROTEIN NITROGEN	
	MACRO-METHOD	MICRO-METHOD	MACRO-METHOD	MICRO-METHOD
	%	%	%	%
Clover roots	1.962	1.972	0.150	0.149
	2.070	2.078	0.121	0.124
	.....	.....	0.113	0.115
	.....	.....	0.102	0.102
	.....	.....	0.094	0.096
Clover shoots	3.209	3.193	0.079	0.079
	2.972	2.961	0.078	0.077
	.....	.....	0.064	0.063
	.....	.....	0.059	0.058
	.....	.....	0.053	0.052
Snap beans	3.560	3.554	0.114	0.114
Snap beans and pods	2.266	2.276	0.073	0.071
Potato tubers	1.448	1.450	0.155	0.156
	1.388	1.363	0.107	0.107
	1.375	1.371	.....	.....
Peas	3.888	3.874	0.275	0.276
	3.897	3.908	0.238	0.237
	3.875	3.874	.....	.....

sample to the second decimal of a milligram. It is, of course, very important that the sample be very finely ground and thoroughly mixed. Since dry tissue is somewhat hygroscopic, it should be allowed to come into moisture equilibrium with the air where the weighings are made. The moisture content of the tissue is determined and the appropriate correction made.

The data in table II indicate that the micro-Kjeldahl method is as accurate as the corresponding macro-method for the determination of total nitrogen or non-protein nitrogen in plant materials. In addition, the micro-method is more economical of material, reagents, and time. The determination may be performed in any laboratory where water and gas are available since no hood is necessary.

The data in table III show that it is possible to employ the micro-Kjeldahl method for estimation of basic nitrogen of plant extracts without digesting the phosphotungstates and filter paper.

TABLE III

COMPARISON OF MACRO- AND MICRO-METHODS FOR THE DETERMINATION OF BASIC AND AMIDE NITROGEN IN PLANT EXTRACTS

RESULTS ARE EXPRESSED AS PERCENTAGES OF THE FRESH WEIGHT

PLANT TISSUE	BASIC NITROGEN		AMIDE NITROGEN	
	MACRO-METHOD	MICRO-METHOD	MACRO-METHOD	MICRO-METHOD
	%	%	%	%
Clover roots	0.061	0.063	0.057	0.056
	0.022	0.021	0.052	0.052
	.....	.....	0.032	0.032
	.....	.....	0.027	0.027
Clover shoots	0.020	0.021	0.065	0.066
	0.010	0.011	0.055	0.055
Potato tubers	0.026	0.028	0.036	0.036
	0.023	0.024	0.033	0.032
	0.020	0.021	0.030	0.031
	0.015	0.015	0.029	0.030
	.....	.....	0.027	0.027

It is also possible to utilize the micro-Kjeldahl equipment for the estimation of amide and ammonia nitrogen of plant extracts (table III). This method cannot be used to determine the free ammonia since a portion of the amide nitrogen is hydrolyzed during the distillation. However, after the amide hydrolysis with H_2SO_4, total amide and ammonia nitrogen were determined just as accurately as in the lengthy aeration method. Recently

PUCHER *et al.* (10) have shown that the conventional methods of determining amide and ammonia in plant extracts may be somewhat in error. Several precautions are necessary in order to distinguish between free ammonia and amide nitrogen. Where ammonia values are low, it is sometimes satisfactory to determine the combined total. In such cases the present method proved valuable though it is recognized that it might be inaccurate with some tissues.

The data in table IV reveal that the amide nitrogen may be accurately determined in 5-ml. aliquots of an asparagine solution as dilute as 0.01 M.

TABLE IV

EFFECT OF DEGREE OF ALKALINITY ON THE DETERMINATION OF AMIDE NITROGEN IN PLANT EXTRACTS AND IN AN ASPARAGINE SOLUTION AFTER ACID HYDROLYSIS
RESULTS ARE EXPRESSED AS MILLIGRAMS OF NITROGEN IN 5 ML. OF SOLUTION

NaOH 30%	Amide nitrogen in 0.01 M solution of asparagine	Amide nitrogen in plant extracts		
		Potato tubers	Clover shoots	Clover roots
ml.	*mg.*	*mg.*	*mg.*	*mg.*
1.8	0.68	0.51	0.27	0.09
2.0	0.68	0.53	0.31	0.08
2.2	0.69	0.54	0.32	0.08
2.4	0.69	0.54	0.32	0.09
2.6	0.69	0.54	0.32	0.09
2.8	0.69	0.53	0.32	0.09
	Calculated 0.70			

The data also show that 2.4 ml. of 30 per cent. NaOH are sufficient to neutralize the acid used for hydrolysis and to furnish sufficient alkalinity for removal of the ammonia in a distillation period of 5 minutes. Lesser amounts of alkali yielded slightly erratic results while larger amounts resulted in no greater yield of ammonia.

Summary and conclusions

The PREGL micro-Kjeldahl method has been applied to the determination of total organic nitrogen in plants. Modifications necessary for making micro-determinations of nitrogen in plant materials are discussed. Micro-methods are proposed for the determination of non-protein, basic, and amide nitrogen in plant extracts.

It is concluded that micro-methods are rapid, economical, and as accurate as macro-methods for the determination of total organic nitrogen in plant tissues and non-protein, basic, and amide nitrogen in plant extracts.

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RATE OF ADJUSTMENT OF LEAF TEMPERATURE TO INCIDENT ENERGY

CHARLES A. SHULL

(WITH TWO FIGURES)

Introduction

The variability of leaf mass as a factor in the energy relations of leaves was discussed (**4**) in a previous paper. The problem was considered with reference to the thermal effects of absorbed radiant energy, especially in the absence of energy loss through transpiration. Data were tabulated to show the specific variability in the mass of one cm.^{2} of leaf tissue, and the corresponding variations in the calculated rates of leaf temperature increase. These calculations were based primarily upon certain fundamental measurements of solar energy receipt, energy absorption by leaves, thermal emissivity of leaf substance, and specific heat of leaves made by BROWN and ESCOMBE (**1**) and BROWN and WILSON (**2**) more than thirty years ago.

In these data there is the implication that the rate of adjustment of leaf temperature to inflowing radiant energy would also vary with the leaf mass, and that the rate of thermal adjustment would be very rapid. This particular phase of the problem was not emphasized in the former paper, and it seems appropriate to call attention to the rapidity of adjustment that should be expected if the leaf is confined to thermal emissivity as a method of energy dissipation, and if the values for incident energy and absorption previously suggested (**4**) are accepted as average for thin leaves.

As was indicated in the discussion of the mass factor, and also in an earlier discussion of the reflection of light from leaf surfaces (**3**), there are important reasons for modifying the values for incident radiation and absorption obtained by BROWN and his coworkers. Their figure for the calorie value of sunlight is considerably above the average value shown by their own measurements; and their coefficient of absorption included the reflected energy. It is now certain that this reflected energy is much too large to be neglected in such calculations.

If one employs the lower values suggested (**4**) for energy receipt ($R = 0.55$) and absorption ($a = 0.58$), retains BROWN's values for the specific heat ($s = 0.879$) and thermal emissivity of leaf substance ($e = 2 \times 0.0366$ cal./cm.^{2}/min./deg.), and applies these values to the leaves of plants listed in table I, the calculated rates of leaf temperature change are much reduced from those published several years ago (**4**). In column 3 of table I are shown the calculations (Ra/ms) of the temperature increase per minute in leaves exposed to radiation of 0.55 cal./cm.^{2}/min., with absorption of 58

per cent. of the incident energy, and with no energy loss except thermal emissivity.

TABLE I

THERMAL ADJUSTMENT OF LEAVES TO ABSORBED RADIANT ENERGY

SPECIES	MASS PER CM.^{2}	*Ra/ms*	TIME REQUIRED FOR THERMAL EQUILIBRIUM
	gm.	*°C.*	*sec.*
Galinsoga parviflora	0.0137	26.5	10.0
Ambrosia trifida	0.0142	25.5	10.4
Ailanthus glandulosa	0.0152	23.9	11.0
Catalpa bignonioides	0.0163	22.3	11.8
Ulmus americana	0.0174	20.9	12.6
Populus deltoides	0.0197	18.4	14.3
Helianthus annuus	0.0212	17.1	15.4
Clematis paniculata	0.0223	16.3	16.2
Geranium sp. (*Pelargonium*)	0.0266	13.6	19.4
Ginkgo biloba	0.0278	13.1	20.1
Syringa vulgaris	0.0317	11.4	23.1
Impatiens balsamina	0.0379	9.6	27.5
Ceratozamia mexicana	0.0407	8.8	30.0
Nelumbo lutea	0.0520	7.0	37.7
Begonia sp.	0.0548	6.6	40.0
Iris germanica	0.0808	4.5	58.7
Crassula arborescens	0.3037	1.2	220.0
Aloe arborescens	0.9465	0.38	695.0

The calculated time required in each case for the leaf to reach thermal equilibrium with the environment, through thermal emissivity as sole means of energy dispersal, is shown in column 4 of table I. Thermal emissivity alone should dissipate all of the energy as fast as it is received when the leaf temperature is 4.4° C. above that of the surrounding air. In the case of *Galinsoga parviflora* the leaf temperature should rise at the rate of 26.5° C. per min., and reach the 4.4° dynamic thermal equilibrium temperature in about 10 seconds. It is seen from the table that all thin-leaved plants should become adjusted to changes in energy receipt with similar rapidity. The more massive the leaves, the slower the rate of adjustment. *Ceratozamia* requires 30 sec., *Iris* almost 1 min., *Crassula arborescens* nearly 4 min., and *Aloe arborescens* more than 11 min. to make a change of 4.4° C. under receipt of 0.319 cal./cm.^{2}/min.

While it has been observed by various investigators that temperature adjustments are rapid in the tissues of stems and leaves, these calculated values are challenging because of the very short time indicated for the attainment of thermal equilibrium under a given change in energy receipt. As the

writer has previously intimated, the energy relations of leaves are in need of reinvestigation. All of the factors involved in energy receipt, energy utilization, and energy loss need to be remeasured with the best precision instruments available. The observations recorded here point to the same conclusion.

Measurements

After making the calculations recorded in table I it seemed desirable to make some observations to determine whether calculated and observed values would be in agreement. The measurements of the changes in leaf temperature following changes in energy receipt were made with thermocouples. Three thermocouples were constructed[1] of no. 28 constantan wire and no. 30 copper wire wound together, flattened, soldered without flux, and connected in series. The cold junctions were immersed in a mixture of ice and water in a thermos bottle, while the hot junctions were inserted through the leaf, with the flat side of the couples held against the lower surface of the leaf as is shown in figure 1. The incident radiation was allowed to strike

FIG. 1. Method of insertion of thermocouples.

the upper surface of the leaf directly over the thermocouples. It was found that puncturing the leaf in this manner did not affect the temperature readings. Measurements made by holding the thermocouples against the lower surface of an unpunctured leaf were the same as those made with the couples inserted through the leaf and touching the lower leaf surface.

The thermocouples were calibrated carefully by connecting them with a sensitive galvanometer, placing the hot junctions in distilled water of known temperature, and noting the readings on the galvanometer scale. The calibration data are given in table II; from these data the curve in figure 2 has been plotted.

The results subsequently obtained with these calibrated thermocouples were always consistent, and readings could be duplicated at will. An attempt was made to measure leaf temperature changes in direct and diffuse sunlight. It was found to be impractical to measure the temperature changes under these conditions, however, because of the rapid fluctuations in the intensity of natural light.

The measurements recorded in tables III and IV were made in a constant temperature room, using light from a 60-amp. "sunshine" carbon arc. Dur-

[1] The writer is indebted to Dr. J. W. MITCHELL and Dr. KARL C. HAMNER for preparation of the thermocouples, and for measurements of the temperature changes recorded in tables III and IV.

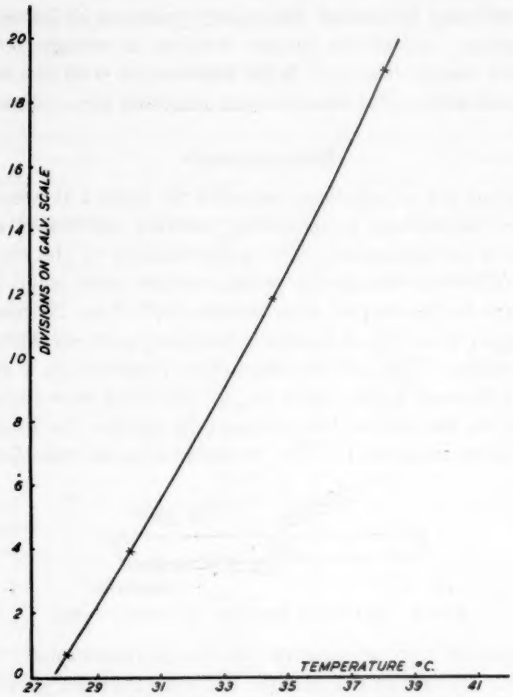

FIG. 2. Calibration curve of thermocouples used.

TABLE II

CALIBRATION OF THERMOCOUPLES

KNOWN TEMPERATURES	GALVANOMETER SCALE
°C.	cm.
28.0	0.7*
30.0	3.6
34.4	11.8
38.0	19.0

* With the cold junction in ice and water mixture, and the hot junctions in water at 28° C. the galvanometer scale was adjusted to read 0.7 cm.

ing the measurements the leaves remained attached to the plants, which were placed at a distance of 2.5 ft. from the arc. The leaves were oriented to receive the incident radiation normal to the upper surface. The galvanometer readings for leaves of the common geranium (*Pelargonium* sp.) *Begonia* sp., and *Caladium neoguineensis* exposed to the diffused and direct radiation

from this arc are shown in table III. The length of time required for the thermal adjustment of these leaves in each case is shown in column 4.

In table IV the galvanometer readings have been converted into temperature readings from the calibration data of table II. From the calculations recorded in columns 2 and 3 it is seen that the temperature changes for the geranium leaf range from 3.2° to 3.6°, averaging 3.4° for five readings. It should be noted that in diffuse light the leaves were about 2.1° cooler than the surrounding atmosphere. When thermal equilibrium had been attained they were 1.3° warmer than the air of the room. It should also be noted in table IV that when the thermocouples were exposed to direct

TABLE III

GALVANOMETER READING WITH LEAVES OF *Pelargonium*, *Begonia*, AND *Caladium*

PLANT	READING IN DIFFUSED LIGHT	READING IN DIRECT LIGHT	TIME REQUIRED FOR THERMAL EQUILIBRIUM	
	cm.	*cm.*	*min.*	*sec.*
Geranium (*Pelargonium* sp.)	4.0	10.3	1	45
	5.0	11.1	1	20
	4.5	11.0	1	30
	5.5	11.3	1	20
	5.2	11.1	1	30
			Ave. 1	29
Begonia sp.	4.8	8.5	2	0
	4.5	8.5	2	0
	3.5	10.0	2	0
	5.8	10.8	2	0
Caladium neoguineensis	9.5	21.0	2	0
	9.8	20.3	1	45
	9.5	19.8	2	0

illumination at a distance of 2.5 ft. from the arc they recorded a temperature 1° higher than when exposed to the diffused light of the arc at the same distance.

The *Begonia* leaves, more heavily cutinized, were slightly above the temperature of the room in diffused light, but averaged only 0.3° above air temperature. When measured in direct light, the temperature rose to 3° above room temperature, a total change of 2.7° C. The *Caladium* leaves showed a larger response than the other leaves. They were 0.5° above the air temperature in diffused light, and increased to 6.0° above the room temperature in diffused light. The total change was 5.5° C. It is not known whether the fact that the thermocouples were placed in contact with the under side of a white area of the leaf had anything to do with the greater temperature change in these leaves.

TABLE IV

TEMPERATURE EFFECTS, DIRECT ILLUMINATION OF LEAVES OF *Pelargonium*, *Begonia*, *Caladium*

PLANT	LEAF TEMPERATURE IN DIFFUSED LIGHT	LEAF TEMPERATURE IN DIRECT LIGHT	TIME REQUIRED FOR THERMAL EQUILIBRIUM	ROOM TEMPERATURE	
				DIFFUSED LIGHT	DIRECT LIGHT
	°C.	°C.	min. sec.	°C.	°C.
Geranium (*Pelargonium* sp.)	30.1	33.7	1 45	32.7	33.7
	30.7	34.1	1 20		
	30.4	34.0	1 30		
	31.0	34.2	1 20		
	30.8	34.1	1 30		
Begonia sp.	30.6	32.7	2 0	30.2	31.2
	30.4	32.7	2 0		
	29.8	33.5	2 0		
	31.2	33.9	2 0		
*Caladium neoguineensis**	33.2	39.0	2 0	32.7	33.7
	33.3	38.7	1 45		
	33.2	38.5	2 0		

* The couples were inserted in the *Caladium* leaves in such a way as to bring them into contact with the lower surface of a white area of the leaf.

In column 4 of table IV it is shown that the geranium leaves required on the average 1 min. 29 sec. for the thermal adjustment to incident radiation, the *Begonia* leaves 2 min., and the *Caladium* leaves 1 min. 55 sec.

The radiation of the arc used on these leaves was measured for its light intensity in foot candles with a Macbeth illuminometer. The readings with normal incidence at 2.5 ft. from the arc averaged about 850 foot candles, approximately one-eighth of the intensity of full sunlight on a clear summer day. The total energy content of the radiation was measured with a calibrated thermopile[2] and Leeds & Northrup potentiometer. The average energy content at 2.5 ft. from the arc was found to be 0.395 calorie/cm.^{2}/min. The total value for sunlight average has been taken in the calculations of table I as 0.55 cal./cm.^{2}/min. The arc possesses about 72 per cent. of the total energy of sunlight, but only 12.5 per cent. of its illuminating power at the distance used.

If we assume that the percentages of reflection and absorption of the arc radiation are approximately the same as for sunlight, $a = 0.58$, the geranium leaf of table I should receive $0.385 \times 0.58 = 0.229$ cal./cm.^{2}/min. Then

[2] Obtained through the courtesy of Mr. ROBERT B. WITHROW, Department of Horticulture, Purdue University.

$Ra/ms = 9.8°$ C./min. At this rate, to make the adjustment of 3.4° C. found by observation in table IV should require only 21 sec.; but the time actually required was 89 sec. (table IV).

The rate of temperature adjustment of the *Begonia* leaf calculated in the same manner should be 4.75° C./min. To produce the observed rise of 2.7° would require 34 sec.; but the actual time requirement was 120 sec.

As mass data are lacking in table I for the *Caladium* leaf, calculations cannot be made of its theoretical rate of adjustment. But from the two examples given, it seems probable that the actual rate of adjustment of leaves to changes in energy receipt is considerably slower than would be expected from calculations based upon such measurements of the factors as have been made.

Discussion

No attempt was made to restrict the leaves to thermal emissivity as a means of energy dispersal. They were transpiring during the measurements, but the transpiration was not measured. If there had been no transpiration, the temperature increase should have been greater than was observed. Under these circumstances thermal adjustment would have taken longer, and the discrepancy between observed and calculated rates would have been greater. It seems, then, that we need a thorough reinvestigation of all the internal and external factors concerned with the energy relations of leaves. Specific leaves should be used since the factors vary with the different species examined. It ought to be possible to obtain a set of values for reflection, transmission, absorption, transpiration, thermal emissivity, respiration, photosynthesis, specific heat, leaf mass, and energy content of radiation, the use of which in calculations would yield agreement with observed leaf behavior during energy receipt. The energy relations of leaves will not have reached a satisfactory status until such agreement between theoretical and observed values has been attained.

The thermocouple measurements of the changes in leaf temperature reported in this paper seem not to be vitiated by poor contact. Careful tests were made which showed that the contacts were close enough to yield accurate readings. The problem of lag in the apparatus was also checked. When the thermocouples were first immersed in distilled water at 30° C., and then suddenly transferred to distilled water at 34° C., the time required to record the change in temperature was 3 sec. And if a leaf were first allowed to reach thermal equilibrium in direct light, and the couples then applied in the usual manner to the under surface of the leaf, the apparatus recorded the correct temperature of the leaf in 2 or 3 seconds after the contact was made. It is evident, therefore, that lag in the apparatus is not suffi-

cient to cause appreciable errors in determining the actual rate of leaf adjustment to changes in the amount of incident radiation.

It is believed that when all of the factors involved in the energy relations of leaves have been correctly evaluated under controlled conditions, calculated and observed behavior will reach close agreement.

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PREPARATION OF SINTERED PYREX GLASS AERATORS FOR USE IN WATER CULTURE EXPERIMENTS WITH PLANTS

A. H. Furnstal and Burkett Johnson

(WITH FOUR FIGURES)

In growing plants under water culture conditions it is frequently important, and sometimes indispensable, to pass air under pressure through the solutions. Such aeration has a profound influence on root development. A practical problem arises of providing means for the uniform distribution of air through the culture solution, especially when tanks or other large containers are employed. In some experiments it is also necessary that the aeration devices shall not yield impurities to the solution; for example, in the study of the effects of elements required by the plant in minute quantity.

It has been found essential to the work of this laboratory to provide extensive facilities for aeration in the greenhouse. Several methods of distributing air were devised but were eliminated for various reasons. Among the devices used were surgical rubber tubing; porous porcelain, alundum, and block tin tubing; Folin tubes; and carborundum blocks. Surgical rubber tubing with a soft smooth wall, pierced with fine needle holes, aerated solutions fairly well, but was discarded because of its rapid deterioration, uneven aeration, and compositional impurities. Block tin tubing, drilled with small holes, was rejected because of uneven aeration and possible toxicity. The principal objections to alundum, porcelain, and carborundum were mechanical unsuitability and possible presence of impurities, both in the aerators and in the connections necessarily employed.

Since inert, resistant units of uniform quality[1] and capable of dispersing the air in fine bubbles were essential, pyrex glass was finally chosen as a suitable medium through which air could be distributed without encountering the objections mentioned. Glass aerators of the type needed were not obtainable on the market, and as methods described in the literature were not applicable to our requirements, it was necessary to develop one.

Many modifications of sintered glass aerators were tried, such as sintered disks sealed into glass tubing of various sizes and shapes, and tubing constructed entirely of sintered glass. Individually these were satisfactory in operation, but could not be economically produced in large quantities and it was difficult to obtain even aeration throughout a group of aerators. The method finally devised incorporates the sintering and sealing in one operation. The resulting aerator is efficient, rapidly and easily made in large quantities, and readily cleaned.

[1] Certain types of glass contain substances such as zinc, etc., undesirable for certain experiments.

Several steps were involved in the production of these aerators, namely, grinding the glass, grading it into sizes by elutriation and sifting, preparing a suitable receptacle, sintering the ground glass within the receptacle, and finally calibrating the sintered elements.

Grinding was accomplished by breaking pyrex glass in an iron mortar; grinding in an ordinary coffee mill equipped with steel plates, until the maximum diameter of the particles was about 1 mm.; and finally, reducing it to a still greater state of subdivision in a steel ball mill, designed in this laboratory (**1**). Portions of 100 gm. each were milled for a period of two hours, using $\frac{3}{4}$-inch steel ball bearings. Particle size varies with the amount of glass taken, the size of the mill, the diameter and weight of the balls used, and the time of grinding.

The powdered glass was then washed for three hours in an hydraulic elutriator (**2**) as suggested by Professor KIRK, of the Division of Biochemistry, and modified in our laboratory. The rate of water flow through the elutriator was controlled by means of a mercury manometer. When water was passed through at a rate of approximately 300 cc. per minute, the glass particles taken from the different compartments were found to be graded into sizes suitable for general use. When experiments necessitated the use of more closely graded aerators, however, it became essential subsequently to sift the glass mechanically into more uniform size. The glass was then treated with hot 15 per cent. sodium hydroxide solution, washed with water,

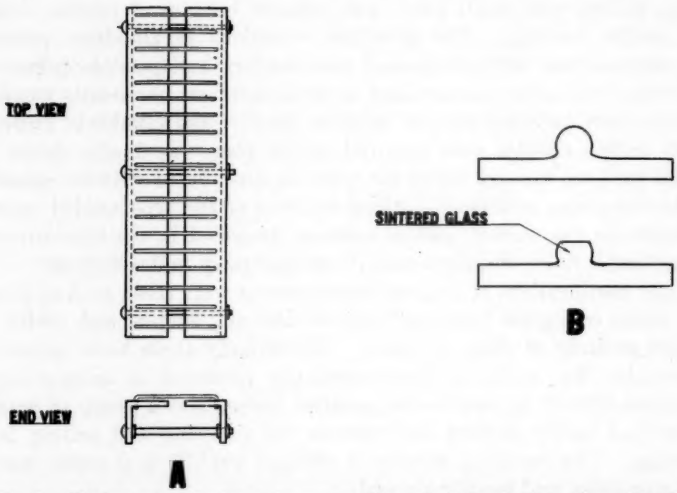

FIG. 1. Equipment and stages in preparation of sintered glass elements: *A*, rack for holding elements during sintering; *B*, glass elements showing bulb receptacle and completed unit.

digested on a steam bath with 40 per cent. (by volume) hydrochloric acid to remove iron particles and other impurities derived from the grinding process, and finally rewashed with water.

The receptacles were made from short lengths of 7-mm. pyrex tubing, a thin-walled bulb being blown in the side as shown in figure 1, *B*. A thin-stemmed funnel was used in filling the bulb, the glass being slightly heaped up to compensate for contraction during sintering and to increase the mechanical strength of the element. The powdered glass was settled in the receptacle by tapping the end of the tube. These tubes were placed, bulb down, on a black iron rack constructed of angle iron (fig. 1, *A*), the rack having previously been dusted with powdered talc to prevent adhesion between the iron and the tubing.

After the muffle had reached the proper temperature, the rack of units was inserted. The temperature immediately fell about 50° and approximately one-half of the total time of sintering was required for the muffle to reach again the initial temperature. The temperature was then held within 2–3 degrees throughout the remaining period, and the rack withdrawn. The total time of sintering, from the time of insertion, was 15 minutes.

The sintering temperatures for the three general elutriator sizes are 770°, 780°, and 790°, for the 40, 45, and 60-mm. compartments respectively

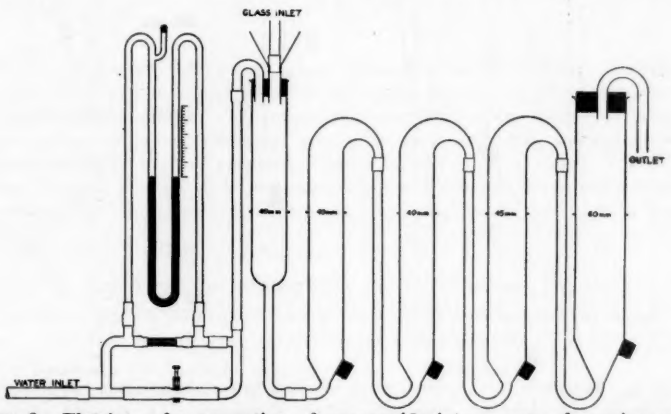

FIG. 2. Elutriator for separation of suspensoids into groups of varying particle size. Suspensions may be removed at desired intervals through the stoppered outlets at the bottom of each compartment, which are 30 cm. in height.

(fig. 2). The porosity, particle size, and sintering temperature of the graded aerators are related in the manner indicated in the following table. Upon cooling, the tip of the bulb was removed on a fine carborundum or emery wheel.

PARTICLE SIZE MESH PER INCH	AVERAGE POROSITY (CM. H_2O)	TEMPERATURE (°C.)
170–200	100	772
150–170	90	780
120–150	80	787
100–120	70	793
80–100	60	800

Sintered glass disks may be prepared in a similar manner, using a talc (soapstone) mold of suitable size equipped with a cover of the same material. The time of sintering in this case, however, must be increased to 20 minutes (for ¾-inch diameter disks).[2] These disks may be sealed in tubing of appropriate size and used where large quantities of air must be distributed (**3, 4, 5**).

Individual aeration elements were calibrated to an arbitrary rate standard of air flow. They were classified in average porosity according to the

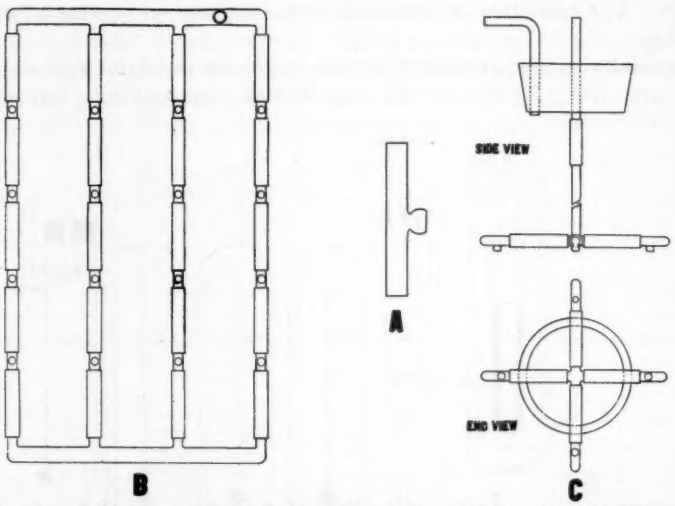

FIG. 3. Adaptations of sintered glass elements to certain aeration requirements for plant cultures (all parts are glass with rubber connectors): *A*, sintered element; *B*, assembly for use in aerating sets of plants during preliminary growth period or in subsequent experimental absorption periods; *C*, assembly for use in bottles or jars, in closed systems. This arrangement has been used effectively in respiration-absorption studies with excised root systems.

[2] An open brass mold dusted with powdered talc has recently proved itself more advantageous.

pressure in centimeters of water required to give the desired rate of flow. This method was found to be sufficiently accurate for most purposes, but when several aerators were to be operated in parallel, they were matched by a substitution method, the aerators being taken from a single calibrated group.

Aerators may be placed in series of multiples, depending upon the type of container used (figs. 3, 4). For special experiments such as those

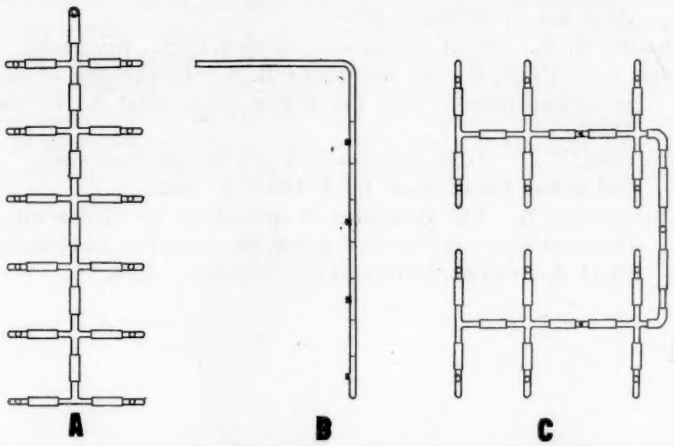

FIG. 4. Adaptations of sintered glass elements to certain aeration requirements, particularly plant culture experiments during growth periods: *A*, 12 unit assembly of glass and rubber connectors. This arrangement was designed to operate in tanks containing 12 plant groups, each effectively aerated by its corresponding underlying aerator; *B*, all-glass assembly composed of elements sealed to each other in series. This arrangement was designed for special plant culture studies where rubber or other impurities might vitiate the conclusions; *C*, sketch showing adaptability of units to multiple assemblies.

involving the use of zinc- or copper-free culture solutions, the glass aerators were sealed to pyrex tubing of the same diameter and bent to fit the container. (All rubber tubings tested in this laboratory were found to contain variable amounts of zinc and possibly other impurities.)

Precipitates formed in culture solutions may cause the rate of aeration to diminish or finally to cease entirely. When thus clogged, the aerators may be cleaned by brushing, or, in extreme cases, by digesting in warm dilute hydrochloric acid.

Aerators of this type have been in continuous use for more than a year and many are still giving satisfactory results.

The writers wish to express their appreciation to Professor D. R. HOAGLAND and Mr. T. C. BROYER for suggestions received.

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BREAKDOWN OF FRUIT AND VEGETABLE TISSUE DUE TO AN ELECTRIC CURRENT

WILLIAM SEIFRIZ

(WITH TWO FIGURES)

Experiments in electrodialysis involving the use of natural membranes revealed the interesting fact that the tissues of fruits and vegetables break down very rapidly when they are the cathode of an electric circuit, but do so only to a slight degree, if at all, when they are the anode.

An apple resting in a bowl containing a weak salt solution, with one electrode of an electric circuit penetrating it and the other electrode projecting into the surrounding solution, will "rot" after eighteen to twenty-four hours if the apple is the cathode or negative pole (fig. 1). If the apple is

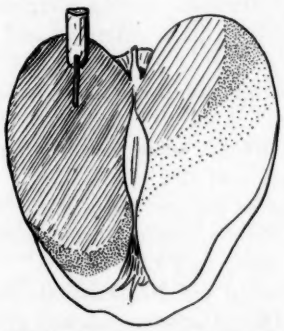

FIG. 1. Effect of the negative electrode of a 110-volt direct current on an apple standing in 0.1 N NaCl for twenty hours. The cross-lined portion is well "rotted" (soft and dark brown in color); the heavily stippled region is considerably discolored (to green), and the lightly stippled portion is slightly discolored.

the anode or positive pole, the tissue is little damaged. The foregoing was the original experiment; it was repeated many times and then duplicated with other fruits and vegetables, always with the same result, except that the degree and kind of degeneration of tissue varied with the material. Particularly striking were the results obtained with the persimmon and potato.

The experimental findings are listed below. The electrodes were of platinum and the circuit a 110-volt direct current, with an intervening 0.5 ampere electric-light bulb. The surrounding solution was 0.1 N NaCl, and the length of the run, eighteen to twenty-four hours. The fruit was immersed in the solution one-half to two-thirds of its height. The degree of acidity of the normal fruit and of the tissues surrounding the respective

poles was obtained by macerating the tissue in a minimum amount of water. The values, in terms of pH, are given, that of the normal fruit first, the treated last. The word "rotting" will be used as a convenient term to express a breakdown or change in the tissue without in any way suggesting the true nature of the change.

APPLE (pH 3.6)—*Cathode:* pronounced rotting; tissue soft and discolored (dark brown); skin turns green; tissue basic (pH 12.2).

Anode: slight rotting; tissue soft but not much discolored; skin turns pink; tissue acidic (pH 3.2).

The experiment was varied by placing the electrodes in separate apples resting in the same solution, and by placing both electrodes in opposite sides of the same apple; the results were identical in all three cases.

PEAR (pH 5.2)—*Cathode:* severe rotting; tissue basic (pH 12.2).

Anode: slight softening; tissue acidic (pH 3.6).

PERSIMMON (pH 5.6)—*Cathode:* rotting complete; tissue becomes black, exceedingly soft, gelatinous in part, collapsed and much shrunken; basic (pH 12.4).

Anode: slight softening and discoloration; acidic (pH 3.0); otherwise normal.

ORANGE (pH 3.8)—*Cathode:* some discoloration and softening; tissue somewhat gelatinous; basic (pH 12.2).

Anode: no pronounced change; tissue slightly more acidic (pH 3.0).

BANANA (pH 5.2)—*Cathode:* rotting throughout the greater part of the fruit; color nearly black; basic (pH 12.2).

Anode: slight softening and discoloration; tissue acidic.

OSAGE ORANGE (pH 3.2)—*Cathode:* marked gelatinization; basic (pH 12.2).

Anode: tissue not noticeably altered; acidic.

ONION (pH 5.4)—*Cathode:* gelatinization of tissue; basic (pH 12.2).

Anode: slight softening; acidic (pH 3.0).

CARROT (pH 4.8)—*Cathode:* marked rotting, discoloration and shrinkage; basic (pH 12.0).

Anode: tissue normal, no noticeable change; acidic (pH 5.3).

POTATO (pH 6.2)—*Cathode:* complete gelatinization of half of the potato (fig. 2); basic (pH 12.0).

Anode: slight softening; otherwise unaffected; acidic (pH 5.4)—(Sweet potato reacts in a manner similar to white potato.)

These results indicate that high alkalinity is constantly associated with breakdown in fruit and vegetable tissue due to the passage of an electric current.

The negative pole or cathode of an electric circuit is alkaline where electrolytes are involved. This is true because the positive ions of alkali metals

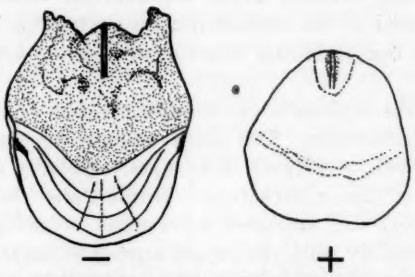

FIG. 2. Effect on potatoes of the negative (left) and the positive (right) electrodes of a 110-volt circuit. The potato (left) in which the cathode was imbedded has become a soft and glutinous jelly throughout more than half its volume (there is no discoloration), while the potato (right) containing the anode was but very slightly affected.

wander to the cathode and there unite with the dissociated hydroxyl ions of the water to form a base. Alkalinity at the cathode may be proved experimentally by adding phenolphthalein to an electrolytic solution into which two electrodes project; the color changes to pink at the negative pole.

As the negative pole is alkaline and as decomposition of the tissues took place at this pole, it is possible that alkalinity *per se* or some reaction which is accelerated by alkalinity, is responsible for the decomposition. It is particularly significant that in all fruits and vegetables with but three exceptions, the pH of the tissue at the cathode was 12.2; in two cases it was 12.0, and in one case 12.4, while the original pH values of the tissues differed considerably.

In addition to alkalinity the causative agent may be the electric current as such, heat, bacteria, oxidation, or autolysis. The electric current probably functions only through some condition which it establishes, such as reduction. Heat was given special consideration because the fruit and vegetables often rose to quite high temperatures owing to high resistance of the tissues, but as the tissue at both positive and negative poles became hot, and as heat applied separately caused no breakdown in tissue, heat cannot be a primary cause. As for bacteria, the reaction is too rapid for them alone to be responsible.

It appears that the reactions which most probably cause a breakdown in tissue due to a flow of current are oxidation and autolysis (proteolytic self-digestion). The alkaline condition developing at the cathode might be expected to hasten non-enzymic oxidations, as there are many instances in which alkalinity is known to promote oxidation; for example, a metal bucket rusts more rapidly when lime is left within it; pyrogallol oxidizes more readily in the presence of alkali. However, oxidation (involving oxy-

gen) as the primary cause of initial degeneration seems unlikely because no oxygen is formed at the cathode during electrolysis. There is also no reason to believe that oxidizing enzymes are rendered specially active at the cathode.

A more probable explanation of the effects observed is suggested by the nature of the degeneration. The tissue tends to become soft, which is a result to be expected from autolytic changes, especially proteolysis, involving breakdown of tissue structure. Intracellular proteolytic enzymes (papain, cathepsin) are activated by various reducing substances, and autolysis sets in rapidly when the oxygen supply to tissues is cut off. Thus, reducing conditions generally seem to favor autolysis or proteolytic breakdown, and the cathode, where electrons are liberated, is the electrode where reducing conditions would be expected to prevail. Proteolysis, therefore, is possibly the initial reaction.

Following this self-digestive breakdown of the tissues, various autoxidizable substances would be liberated, and atmospheric oxygen would penetrate more easily. The coloration observed might then be due to a secondary reaction involving oxidations catalyzed by oxidases, with still further oxidation by H_2O_2 (formed as a by-product) catalyzed by peroxidase. The substances oxidized by H_2O_2 and peroxidase are all phenolic (gallic acids, tyrosine, catechol, etc.) and the oxidation products are almost always colored. (According to BALLS and HALE,[1] the surface darkening of fruits is catalyzed largely by peroxidase, a preliminary step being the formation of hydrogen peroxide by a respiratory enzyme.)

If the strongly alkaline condition near the electrode develops immediately and rapidly, it is unlikely that catalysis is responsible for the changes in the tissue because enzymic reactions could not take place. The changes might then be due simply to alkaline hydrolysis of the cell materials. Such would be true in the case of proteins and amino acids. Proteins on hydrolysis yield amino acids of which tyrosine is one of the most abundant. An oxidation product of tyrosine is quinone which is of brown color. Pectins may also be involved; they form pectic acid and methyl alcohol in the presence of dilute alkalies. Starches, however, are very stable even in the presence of dilute alkali. Alkaline hydrolysis of proteins may, therefore, be responsible for the breakdown of fruit and certain vegetable tissues, but this cannot be the cause of the change in the potato in which starch is primarily involved and where no discoloration takes place. We return, therefore, to the possibility that the high alkalinity observed develops only after the tissue is already partly liquefied and the current flowing strongly, in which case autolysis (enzymic proteolysis) could take place.

[1] BALLS, A. K., and HALE, W. S. Peroxidase in the darkening of apples. Ind. & Eng. Chem. 27: 335–337. 1935.

Certain features of the experiments here described appear to be new, certain others have an historical background. In 1791 GALVANI[2] made the observation that experimental animals which have been previously subjected to electrical treatment, spoil much faster than those not so treated. Later, HUMBOLDT[3] reported some observations of BENJAMIN FRANKLIN, who, he says, was the first to remark that putrification takes place very quickly in animals or parts of animals in which the irritability has been destroyed by strong electrical shock or wasted by repeated contractions. HUMBOLDT adds that he had often made the same observation in regard to animal tissues used in galvanic experiments. The legs of frogs to which a current had been applied for a long time, putrefied several days sooner than those which had not been so treated.

The observations of GALVANI, FRANKLIN, and HUMBOLDT are other instances of the acceleration of the rotting of tissues as a result of the passage of a current. The current as such is not the primary cause of rotting in these historical experiments any more so than in the experiments reported here. That the current itself is not responsible is shown by the observation of FRANKLIN that muscle which has been wasted by repeated contractions also putrefies very quickly just as does muscle which has been subjected to an electric shock.

Summary

1. The tissues of fruits (apple, persimmon, etc.) and vegetables (potato, etc.) are broken down to a soft and usually dark (brown to black) colored mass when they are the cathode of a 110-volt direct electric current. The anode produces little or no effect.

2. All tissues assume a remarkably uniform pH value (of 12.2) at the cathode.

3. The primary cause of the degeneration is thought to be autolysis (proteolysis) catalyzed by intracellular proteolytic enzymes, which are rendered active by the reducing conditions existing at the cathode. Proteolysis is (in most instances) followed by oxidation (and discoloration) which is accelerated by the highly alkaline condition existing at the cathode. These oxidations are catalyzed by oxidases and peroxidases and probably involve phenolic compounds.

4. If the highly alkaline state existing at the cathode is immediately produced, then proteolysis, which would be inhibited by so high a pH as

[2] GALVANI, A. Abhandlung über die Kräfte der Elektrizität bei der Muskelbewegung, 1791. (W. OSTWALD'S Klassiker der exakten Wissenschaften, no. 52, Leipzig, 1804.)

[3] HUMBOLDT, F. A. Expériences sur le Galvanisme, et en général sur l'irritation des fibres musculaires et nerveuses. Paris. 1799.

12.2, probably does not occur; in this case direct alkaline hydrolysis of proteins may be responsible for the breakdown in tissue. It is believed, however, that alkalinity follows proteolytic degeneration.

5. Potato and onion tissue gelatinize at the cathode into a typical jelly, and do not become discolored (oxidized) when traversed by an electric current.

I am indebted to Dr. K. A. C. ELLIOTT for suggestions bearing on the interpretation of the foregoing experimental results.

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NORMAL VIABILITY OF SEEDS AND BACTERIAL SPORES AFTER EXPOSURE TO TEMPERATURES NEAR THE ABSOLUTE ZERO

CHAS. B. LIPMAN
(WITH THREE FIGURES)

In 1934 G. N. LEWIS and the writer[1] reported the results of an experiment in which nine varieties of seeds of higher plants were kept in liquid air for sixty days, after which the seeds were removed and were found to germinate as well as control seeds of the same varieties, which had been kept at laboratory temperatures. It seemed to me desirable also to subject similar seeds, and also bacterial spores, to temperatures as close as possible to the absolute zero, so as to be able to ascertain if an approach to the absolute cessation of molecular motion was or was not differently effective than about – 190° C. Through the kind cooperation of Professor W. F. GIAUQUE, of the Department of Chemistry of the University of California, such an experiment was rendered possible with the use of apparatus employed by Professor GIAUQUE in his well known low-temperature investigations. Accordingly, seeds of the following higher plants were placed in several glass tubes about 1 cm. wide and 10 cm. long, and plugged with cotton: vetch, wheat, barley, *Melilotus*, tobacco, flax, buckwheat, spinach, Milo maize. In similar tubes, fragments of cover glasses upon which there had been smeared suspensions of spores from common spore-forming bacilli, in cultures available in the laboratory, were inserted. Both seeds and spore smears had previously been kept for two weeks in a desiccator over sulphuric acid in partial vacuum.

On the sixteenth of April, 1935, the tubes were placed end to end in a long narrow tube which, in turn, was placed in the low temperature apparatus. After an exposure of less than twenty-four hours, a leak was discovered in the apparatus which made it impossible to attain the desired low temperatures and the run was temporarily discontinued. This incident is mentioned, however, to indicate that some hours of exposure to very low temperatures (how low is not accurately known) was accorded the seeds and spores additional to those definitely given below in the experiment proper. After two or three days the leak in the apparatus was found and stopped, and the experiment started again. This was at 10:00 A. M. of the twentieth of April, 1935. The tube containing the seeds and spores was cooled slowly from room temperature to 4.2° K. at 11:40 A. M. The temperature remained at that point until 1:15 P. M. of the same day and

[1] LIPMAN, CHAS. B., and LEWIS, G. N. Tolerance of liquid-air temperatures by seeds of higher plants for sixty days. Plant Physiol. 9: 392–394. 1934.

then, through application of vacuum on liquid He bath, dropped in less than two hours to 1.35° K. The latter temperature was maintained for a little over two hours, after which the temperature went up to 4.2° K., and was held at that point until 8:00 A. M. on the twenty-second of April, 1935.

After that, the run was stopped and the apparatus allowed to warm up slowly to room temperature. At 10:00 A. M. of the twenty-second, the tubes containing the seeds and spores were removed from the long tube which had held them in the apparatus. There was no noticeable change in the seeds as a result of the exposure to the extremely low temperatures noted. The tubes were taken to the laboratory. The seeds were then strewn on wet filter paper in Petri dishes and covered with more wet filter paper. The

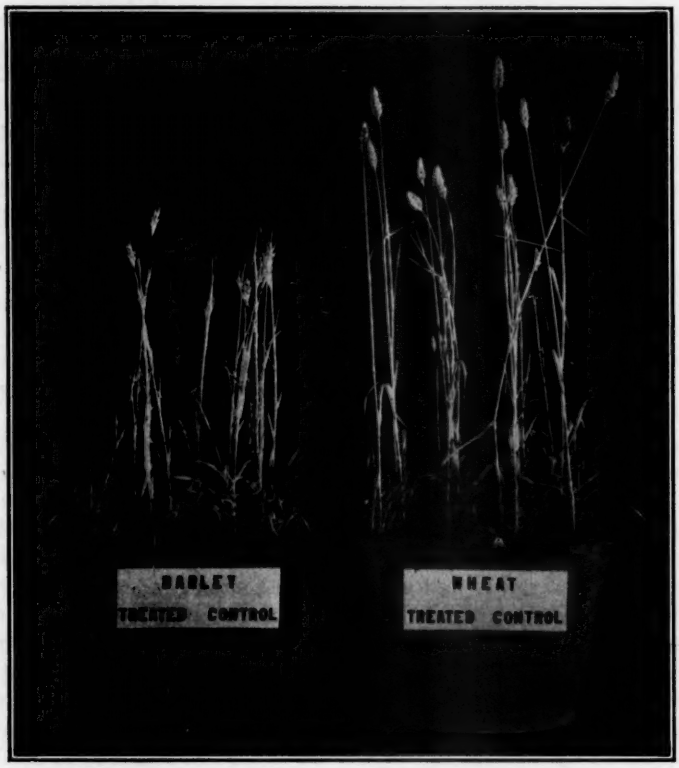

FIG. 1. Left to right: Barley and wheat. The left half of each pot represents growth of seeds kept from about 1° to 4° K. for forty-four hours, while the right half represents growth of control seedlings.

FIG. 2. Left to right: *Melilotus indica* and buckwheat. Legend otherwise as in fig. 1.

Petri dishes were kept in the incubator at about 28° C. After twenty-four hours a few of the seeds had begun to germinate. After forty-eight hours many more had germinated. The control seeds under the same conditions, while showing the same percentage germination, developed more rapidly.

When the seedlings had developed to the age of four days, six of the species were planted in large pots of moderately good soil and placed in the open air outside of the greenhouse. In each pot one-half of the surface was planted with the seedlings from the treated seeds, and the other half with seedlings from control seeds. The pots were regularly irrigated, the number of seedlings thinned to a small number as they increased in size, and allowed to grow to maturity. When mature they were photographed and the photographs accompany this paper. They speak largely for themselves. Only the pot planted to tobacco was not photographed. This was owing to the fact that the control tobacco seedlings died for some undiscovered reason.

It is perfectly clear from the photographs that there is no discernible difference between the plants grown from the treated seeds and those grown from the control seeds. In other words, an exposure of seeds to approximately 1° K. to 4° K. for more than forty-four hours did not destroy the viability of the seeds nor their power to produce perfectly normal plants

which came to maturation equally with control seeds which had received no treatment. The seeds from the plants shown in the photographs are being saved and will be planted for another crop, in order to ascertain what effect, if any, was exerted upon the second generation by exposure of the seeds to temperatures near the absolute zero.

The spores of certain bacilli which were exposed to the low temperature treatment along with the seeds, as stated above, were transferred to sterile soil extract-peptone media in Erlenmeyer flasks. This was done in a sterilized inoculation chamber. Within a few hours very heavy growth was evident in these flask cultures. Microscopic examination of the cultures disclosed the presence of the same rods which had originally produced the spores used in the experiment. The vigor of the growth noted in these cultures indicates that bacterial spores in a dry state (the experimental material was dried along with the seeds) are fully as resistant to temperatures near the absolute zero as seeds.

The foregoing facts render it at least possible that seeds and spores could be preserved indefinitely at or near the absolute zero, at higher temperatures like that of liquid air, and even at higher temperatures than these. I have discussed this subject in the paper previously cited. Be that as it may, the most significant conclusion which flows from the results obtained in this experiment is that living organisms in resting states, such as seeds and spores, do not need to respire in order to maintain the life pattern, nor to retain the power to develop into vegetative forms when the proper environ-

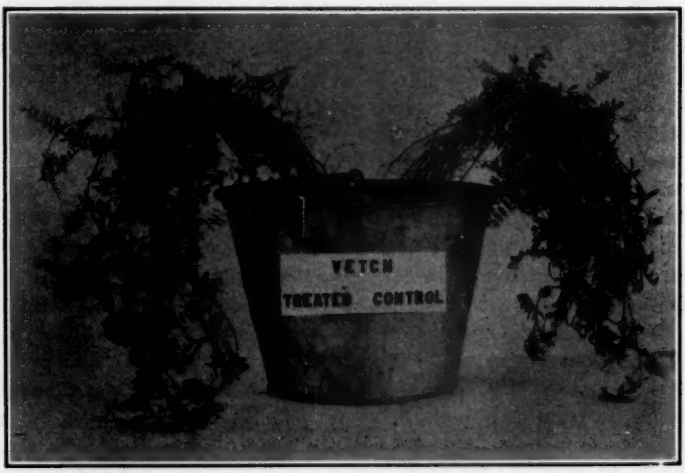

FIG. 3. Vetch. Left half of pot shows seedlings from treated seeds. Right half of pot, control seedlings. See remainder of legend under fig. 1.

mental conditions are supplied. This conclusion is drawn on the basis of the fact that seeds or spores kept at the low temperatures in question cannot have respired; and yet they remained, in the absence of respiration, during protracted periods, fully capable of vigorous development into the respective active forms when moisture and warmth were supplied.

The view here expressed, that living matter may retain all the properties of life without respiring is of course contrary to the established tenets of biology, but it seems to be irrefutably supported by the evidence furnished in this paper and in the paper cited. Moreover, it receives further support from the experiments of Dr. DEAN BURK, as reported by him orally to me. *Azotobacter* cells, in a vegetative form, may be kept for several days in the absence of oxygen without losing their living powers when returned to a proper environment. Moreover, the conclusion here drawn relative to the absence of respiration in seeds and spores which tolerate temperatures close to the absolute zero, is in accord with the experimental facts which I have described in several papers, and with those which I hope to describe in several more, to the effect that at least some bacteria in spore form may live inside of rocks, dry adobe, and many other old materials ranging widely in age, and be capable, under proper conditions, of returning to a perfectly vigorous vegetative state again.

I wish to express my deep appreciation to Professor W. F. GIAUQUE, who cooperated with me so generously, and to Mr. W. C. CHANDLER, for his assistance with the experimental work.

UNIVERSITY OF CALIFORNIA
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BRIEF PAPERS

DIAGNOSIS OF PLANT TROUBLES WITH DIPHENYLAMINE[1]

The testing of a plant part with a sulphuric acid solution of diphenylamine is frequently used as a means of determining a need for nitrogen. The test is interpreted on the basis that one drop of the solution on a cross section of a plant part imparts a blue to almost blue-black coloration depending on whether there is a trace or a relatively large amount of nitrates present. A lack of blue color indicates an absence of nitrates signifying a nitrogen deficiency in the plant and its root environment.[2]

This test for the above purpose has its limitations, but its application may be broadened by knowledge of the plant being tested, including the state of activity of the plant part.

A list of the plants tested follows. No general rule can be given that would indicate the use of a definite plant part. The plants were known to be amply supplied with nitrogen. The diphenylamine solution was made by dissolving 0.2 gm. in 100 cc. of sulphuric acid (c. p.) as used by MORGAN.[3]

Geranium

Positive test at base of petiole and also a cross section of petiole.

Fuchsia

Negative at base and cross section of petiole.

Positive at node, but a stronger positive above and below a node.

Begonia

Positive at base of petiole.

Primula

Positive at base of petiole.

Asparagus sprengeri

Positive where growth is slow.

Negative on new shoots.

Negative on old shoots.

Tomato

Positive at base of petiole.

Rose

Negative where growth is active.

Positive where there is no growth, but where transpiration is taking place.

[1] Contribution no. 229 of the Massachusetts Agricultural Experiment Station.

[2] In the absence of a blue color a brown color may develop due to carbonization of plant tissue by the test solution.

[3] MORGAN, M. F. Microchemical soil tests. Connecticut Agr. Exp. Sta. Bull. 333. 1932.

An actively growing plant part may give a negative test with diphenylamine because the nitrate is being utilized in growth and therefore does not accumulate in tissue contiguous to the region where it is being transformed into protein material. By testing older parts of the plant or sections where transpiration is fairly active and growth has ceased, a positive test may be obtained.

Diagnosis with diphenylamine

The diphenylamine test is useful in problems connected with physiological disturbances and often may give a clue to the cause. Thus in cases where plants suddenly wilt in the presence of an apparently sufficient supply of water with additional symptoms of dying cells between the veins of the leaves, there is the suggestion of salt injury due to misuse of fertilizer. Testing the killed area with diphenylamine either at the time of wilting or several days later has proved useful if nitrate was a constituent of the fertilizer. But a negative test should not deter the investigator from considering non-nitrate fertilizers as exempt in causing the injury. Thus ammonium sulphate may cause the same type of injury, but fail to give a positive test with diphenylamine. The limitations of this test were definitely determined by killing the leaf tissue of geranium plants with heavy applications of sodium nitrate, ammonium sulphate, sodium chloride, formaldehyde, and hot water. The symptoms from the various causes were indistinguishable. The leaf tissue was tested with diphenylamine 48 hours after the chemicals were applied. The plants killed with sodium nitrate were the only ones giving a positive nitrate test. Two weeks after the ammonium sulphate was applied, the leaves did give a positive nitrate test. One could interpret this peculiar result as indicating that there was either a photochemical effect on the ammonium ion in the dead tissue or else nitrification took place in the soil and the nitrates accumulated in the leaf owing to the conducting tissue's remaining intact in spite of the death of the leaf tissues. At no time could positive tests be obtained in the leaves killed by sodium chloride, formaldehyde, or hot water.

Plants bearing small yellow leaves and lacking the softness of tissue associated with vigor suggest a nitrogen deficiency. In two such cases, roses and gardenias, the diphenylamine test showed in the leaves a strong positive test for the presence of nitrates. The disturbing factor causing the yellowing made it impossible for the leaves to utilize the nitrogen. This case is, therefore, one in which a nitrogen deficient leaf has an ample supply of nitrogen present, but it is not assimilable.

Photochemical effect on the ammonium ion

When nitrogen deficient plants are supplied with nitrogen from such inorganic sources as sodium, potassium, and calcium nitrates and ammo-

nium sulphate, and also from organic sources such as urea, dried blood, tankage, fish, and cottonseed meal, the plants will, in 10 to 20 days, assume a healthy green color and respond positively to the diphenylamine test.

Testing for nitrate when the source of nitrogen is from the ammonium ion has given some interesting results even though it is difficult to make a comprehensive interpretation of them.

There have been times when, in a 24-hour period, ammonium sulphate applied to the soil gave positive tests for nitrate in the plant equal to those obtained from sodium nitrate. At other times positive tests were not obtained until 3 days after the ammonium sulphate was applied. In the first case it is doubtful whether nitrification of the ammonium ion by bacteria could take place rapidly enough to account for the nitrate near the top of a tomato plant a foot high. In the second case, in which the time interval was three days, nitrification outside of the plant may have taken place.

Some tests and observations tend to support a hypothesis that there is a photochemical or heliochemical effect which, acting with the aid of plant cells, may cause a transformation of the ammonium to the nitrate ion. There are times when it appears as though sunlight initiates this transformation. At other times it appears that the plant part cannot utilize the ammonium ion at the time being, and therefore some mechanism transforms it to the less injurious nitrate form.—LINUS H. JONES, *Massachusetts Agricultural Experiment Station.*

A DILATOMETER FOR PLANT MATERIALS

(WITH ONE FIGURE)

Several types of dilatometers have been developed to study the changes in volume accompanying imbibition and to measure the rate and amount of hydration in colloidal systems (**1, 2, 3, 4, 5**). The dilatometer which has been used in the plant physiology laboratory at the University of Maryland possesses certain advantages that facilitate its use and increase its accuracy. The details are shown in figure 1.[1] It is an all pyrex glass system, and can be evacuated and filled without disturbing the material used as a sample. The apparatus has proved particularly valuable for accurately measuring changes in volume accompanying imbibition in whole and ground corn kernels, and in demonstrating the condensation of water by plant colloids.

The dilatometer consists of a 250-ml. heavy pyrex Erlenmeyer flask with a ground glass joint between it and a calibrated capillary tube that contains a three-way stopcock. To the bottom of this Erlenmeyer flask is fused a

[1] Constructed by Mr. CLARK, of the Smithsonian Institution, Washington, D. C., under specifications furnished by the writers.

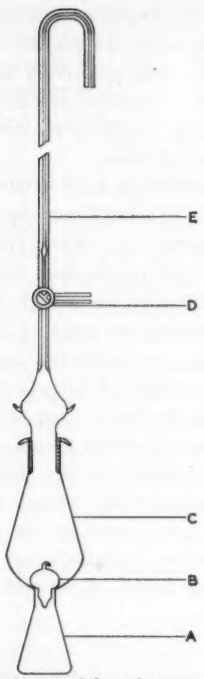

FIG. 1. Dilatometer for plant materials: *A*, mercury well; *B*, weight; *C*, reaction chamber; *D*, three-way stopcock; *E*, capillary tube.

50-ml. pyrex Erlenmeyer flask, which forms the mercury well for the material while being evacuated and thermostated. A pyrex glass bulb filled with mercury acts as a weight to hold the material beneath the mercury. The dimensions given in the preceding description may be modified as the materials or aims of the investigator require.

To operate the instrument, the lower compartment (*A*) is partially filled with mercury. The material being studied is placed on top of the mercury and covered with the weight (*B*). After the apparatus is assembled in place, the entire system is evacuated under a pressure of 0.05 mm. to displace the gases in or on the sample. The time period of evacuation will vary, and must be determined experimentally for each kind of material used. Sufficient mercury to cover the sample is then introduced through the capillary and allowed to trickle around the loosely fitting weight into the lower compartment. The system is evacuated a second time, and distilled water, boiled and cooled to the correct temperature, is admitted through the capillary by adjustment of the three-way stopcock, until the

system is completely filled. The apparatus is now ready to be placed in an accurately controlled thermostat. Observations and readings of the height of the meniscus in the capillary will enable the operator to determine when the system has reached thermal equilibrium. The apparatus is tilted sufficiently to permit the weight to fall away from the mouth of the lower chamber, thus allowing the sample to come into contact with the water. Volume changes can now be recorded.—G. A. GREATHOUSE AND M. W. PARKER, *University of Maryland.*

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CHLOROPHYLLOMETRY

As is well known, chlorophyll is in many respects the analogue of the haemoglobin of the blood of animals. Indeed, it is more than probable that haemoglobin is derived from chlorophyll by the substitution of iron for magnesium. Medical men have long estimated the relative haemoglobin content of the blood by various color matching methods, and haemoglobinometers of several types have been in common use for many years. Haemoglobinometry has been of great value both in clinical pathology and in pure research.

The same principles and simple methods used in haemoglobinometry may readily be applied to the measurement of differences in chlorophyll content in plants. The following is an outline of the adaptation of the methods of haemoglobinometry to chlorophyll estimation. The first step required is the establishment of a color standard. The leaf of the common hard maple, *Acer saccharinum,* was selected as readily available. One third of 1 gm. of the fresh leaf from the blade, but rejecting the midrib, was taken. This was cut up into fine pieces (3 or 4 mm. square) with an ordinary scissors, and immersed for 24 hours in 10 cc. of pure alcohol. At the end of that time the green alcohol was decanted from the leaf sections, the pieces of which now resembled pieces of white paper. The alcoholic extract of the chlorophyll was then placed in an ordinary Dubosecq colorim-

eter and compared with a similar extract from the leaf of another plant. The one first used was from the leaf of a housegrown sweet potato vine. The difference in the chlorophyll content was marked. With the maple leaf as 100, the reading for the chlorophyll content of the sweet potato leaf was 45.

There was now opened up a rapid, simple, and interesting method of quickly making relative studies of the chlorophyll content, not only of the leaves from plants of different families and types but of leaves from different locations on the same plant, from healthy and diseased plants, from different parts of the same plant, and from plants subjected to alterations in environment.

The simplicity of this method enables an investigator to estimate the chlorophyll content of any part of the plant with relative ease and rapidity. It offers an opportunity to add to the knowledge of plants from the standpoint of pure science, but it is almost certain that, like haemoglobinometry, it will also have practical applications.

Studies of relative chlorophyll content of different plant families are now being made and will be the subject of a future report.

In addition, a chlorophyllometer on the plan of the Sahli haemoglobinometer is being designed. This will have a sealed tube containing a diluted standard solution and another open mixing tube for the solution to be tested, the two mounted on a milked glass background and with a suitable scale for reading the percentage of chlorophyll directly, as haemoglobin percentages are read with Sahli haemoglobinometers.—Louis H. Roddis, *Commander, Medical Corps, United States Navy, Washington, D. C.*

NOTES

St. Louis Meeting.—The twelfth annual meeting of the American Society of Plant Physiologists was held at St. Louis from December 31, 1935, to January 2, 1936. The arrangements for meeting places had to be changed at a late date because of the incomplete condition of the Municipal Auditorium where the meetings had been scheduled to be held. The change to the lecture rooms in Duncker Hall, Washington University, made necessary a time consuming transfer between hotels and meetings. In spite of these difficulties the meetings were well attended, and the program as a whole was very satisfactory. The large number of papers offered is a constant challenge to the ingenuity of the program committee. Lists of papers to be read by title only, or a list to be presented only in the form of mimeographed abstracts, but with the privilege of elaboration by the author if called upon by the audience might somewhat relieve the congestion. Why should we not try to find some better method of arranging our programs to avoid the overly long lists of papers?

The annual dinner of the Society at the Statler Hotel, on the evening of December 31, was probably the largest gathering of this nature in the history of the organization, exceeding slightly the large attendance at Pittsburgh last year. The dinner was featured by an address by the retiring president, Dr. BURTON E. LIVINGSTON, and by the fourth STEPHEN HALES lecture by Dr. CHARLES A. SHULL. Following the addresses several corresponding members were elected, and the eleventh award of the CHARLES REID BARNES life membership was announced by Dr. JOHN W. SHIVE, chairman of the award committee. Lighter entertainment from the floor, and singing of popular songs filled in the time until the New Year was officially ushered in.

Annual Meeting, 1936.—As these notes are being prepared, notice has been received that the A.A.A.S. will meet in Atlantic City in 1936, instead of in Washington, D. C. The meeting at Atlantic City in 1932 was a very pleasant one, except for the incessant rains which prevailed at the time. As this is one of the quadrennial meetings, the various organizations should by all means arrange for meetings at the same time and place. Cooperation with respect to the larger and broader aspects of scientific advancement of our nation is necessary, and common meeting places increase the opportunities for such cooperation. The program committees for the Rochester summer meeting, and the annual meeting in December, 1936, will be announced in the April number of PLANT PHYSIOLOGY.

Barnes Life Membership.—The annual award of the CHARLES REID BARNES life membership was made to Dr. FRANK MARION ANDREWS, pro-

fessor of botany in Indiana University, who has been associated with the work and progress of the American Society of Plant Physiologists ever since its organization.

Professor ANDREWS was born at Vienna, Indiana, July 27, 1870. He was educated in the public schools of the state, and received his earlier degrees from Indiana University, A.B. in 1894, and A.M. in 1895. Later he joined that distinguished group of American students who at about the turn of the century went to the German universities for more advanced specialization. It was his privilege to study under the guidance of Professors W. PFEFFER, A. FISCHER, L. JOST, and H. DE VRIES. The Ph.D. degree was awarded at the University of Leipzig in 1902. Afterwards he returned to the European laboratories, visiting Amsterdam in 1907, Leipzig in 1907–1908, and Strassburg in 1908–1909. He occupied the Smithsonian table at the Naples Zoological Station in 1908. His services at Indiana University extend through a period of 42 years, a record of faithfulness with few parallels. He began as an assistant in botany in 1894, and rose progressively through the ranks, instructor in 1897, assistant professor in 1904, associate professor in 1908, and professor since 1922. His contributions to the literature of plant physiology include more than a hundred titles. His favorite fields of research include streaming of protoplasm and other protoplasmic behavior, effects of centrifugal force upon cells, pollen germination, and plant movements.

The society is honored in adding his name to the roster of CHARLES REID BARNES life members.

Corresponding Members.—It is a pleasure to announce that the Society has again selected several outstanding plant physiologists of other nations for election to corresponding membership. Previous to the St. Louis meeting, four corresponding members had been elected. One of these, Professor F. A. F. C. WENT, of the University of Utrecht, died late in July, 1935. In addition to the three previously chosen living members, three more have been elected: Dr. BOHUMIL NEMEC, Charles University, Prague; Dr. NICOLAI ALEXANDROVITCH MAXIMOV, University of Saratov; and Dr. HANS MOLISCH, University of Vienna. These men, whose contributions to plant physiology have brought them world-wide recognition, American plant physiologists are happy to honor in this manner, and to extend to them hearty greetings and good wishes.

Western Section.—The most important organizational progress made by the American Society of Plant Physiologists during the year 1935 is the development of the Western Section. A petition bearing the signatures of about 40 members was presented to the Society, requesting authorization of

the development. The territory included is large, and embraces all states west of, and including, Montana, Wyoming, Colorado, and New Mexico. The Hawaiian Islands are also included in this section. It is expected that the new section will affiliate with the American Association for the Advancement of Science through the Western and Southwestern Divisions of that organization. The officers chosen to guide the destiny of the section during its first activities are as follows: Chairman and representative on the executive committee of the national organization, Dr. D. R. HOAGLAND; Vice-chairman, Dr. J. P. BENNETT; Secretary-treasurer, Dr. H. F. CLEMENTS. It is very gratifying to see this extension of regional activity. If other regions of the United States desire to develop regional activities, interested members are invited to communicate with the officers of the Society. At the St. Louis meeting, it was learned that another national organization had completed the organization of fourteen such regional subdivisions which embraced the entire territory of the United States. Regional organization will be encouraged wherever there seems to be strong enough sentiment to insure success of the regional group. The initiative should rest with the members residing in the region where organization is desired. The Western Section is assured of success from the beginning of its career, and the Society as a whole is greatly strengthened by this development. It is hoped that gradually other regions will emulate the example of the western members. The advantages of such regional organization have been recognized wherever regional sections have been developed.

Manuscripts and Abstracts.—During the last two years there has been a limitation of 20 pages on the length of printed papers in PLANT PHYSIOLOGY, and a strict limitation on tables and cuts. These limitations, having served their purpose, have been somewhat relaxed by action at the twelfth annual meeting of the Society at St. Louis. For the ensuing year, the limit will be set at 24 pages, and tables and cuts left to the discretion of the editorial committee. The requirement of a fee for publication of papers for non-members is retained.

In the interests of economy of time and expense, authors are again requested and urged to retain a carbon copy of the final draft of papers submitted for publication. This copy should be used by the author in connection with the proof reading of the galleys. The publishers send the original manuscript to the editorial office to facilitate the editorial proof reading. The author is expected to read his proofs without the original manuscript before him. The carbon copy is to be used for this purpose.

Authors need to be reminded again of the all too frequent carelessness in connection with literature citations. In some recent papers the editors have found that only a very small number of the citations are without defects of some kind. Carelessness with the references in a paper is indefensible.

The last duty of an author before mailing the manuscript to the editors should be a thorough rechecking of the citations against the originals. It is the only way in which the errors can be eliminated.

Abstracts are needed for Biological Abstracts, and these should be returned when possible with the galley proofs, at the same time that the reprint orders are filed. The work of the editorial offices would be very greatly facilitated if authors would file the abstracts promptly. The author is not expected to be able to give the correct page references in his abstract. The editors add these references after the papers are in page proof. We are confidently asking for full cooperation from all contributing members in regard to the handling of manuscripts and abstracts.

Endowments.—Attention of the members of the Society is called to the endowments maintained by our organization. The largest single endowment is the CHARLES REID BARNES endowment, which at the close of 1935 had reached a total of $3000. The STEPHEN HALES Prize endowment has only $1100. As part of this fund must be reinvested during 1936, presumably at reduced rate of income, it is obvious that this endowment will probably prove inadequate for its purpose following the reinvestment. The Life Membership endowment is now $1100, and the General Endowment was reported by the treasurer as $180.13 at the time of the report, December 27, 1935. These funds are a challenge to the generosity and vision of our members. The activity of the Society in its publication ventures will possibly create some reserves against future needs. But the endowments grow mainly as individuals who see the needs and advantages of trust funds held for specific purposes may find an impelling interest in the increase of these funds. Those who feel a keen sense of pleasure at seeing some fellow botanist honored for his contributions to the advancement of our science must also feel that the accumulation of the funds for these specific purposes has been worth many times the effort and sacrifices involved in their accumulation. The enlargement and maintenance of these funds at a level adequate to produce the necessary income to continue these annual and biennial awards in the face of falling rates of return on investments is one of the problems to be kept in mind.

Reprints.—Contributors residing outside of North America are expected to remit for their reprints at the time the orders are filed. To facilitate matters, a statement of the cost of reprints will be sent to the author as soon as the length of the reprint is known. The type will be kept for three months, during which time the financial arrangements may be completed. In the event that orders are not placed and payments completed within this time limit, the type will be destroyed without the issue of reprints. Gratis

reprints are provided only to authors who order and pay for reprints in addition to those presented by PLANT PHYSIOLOGY.

International Congress Photograph.—We are happy to be able to present in this issue of PLANT PHYSIOLOGY a reproduction of the photograph of plant physiologists who attended the Sixth International Congress of botanists at Amsterdam. The editors of PLANT PHYSIOLOGY are not responsible for the identification of the individuals in the photograph, except that no. 100 has been changed because of an error in the original identification. A key was provided with the original photograph, and this merely has been rearranged to fit to the numbering system used in the reproduction. A few individuals have not been identified. If anyone can identify any of those who have not been named, correct information will be greatly appreciated. Also, if any errors are found in the identifications as given, we hope that such errors may be reported promptly.

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